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Land based testing of the CleanBallast ballast water management system of RWO - Final Report

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– an institute in the Environmental Research Alliance of Norway

REPORT

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Abstract

The CleanBallast ballast water management system of RWO, has been land based tested according to the IMOs *Guidelines for approval of ballast water management systems (G8), Res. MEPC.125(53) Annex 3* and *Procedure for approval of ballast water management systems that make use of active substances (G9) MEPC 57/21 Annex 1, Res. MEPC.169 (57), 2008.* The tests were conducted at NIVA's test site located at Solbergstrand 20 km south of Oslo. Test cycles were conducted with medium and high salinity water. Each cycle lasted for a period of 5 days, and a total of 13 test cycles have been completed.

4 keywords, Norwegian	4 keywords, English
1. Ballastvann	1. Ballast water
2. Toksikologi	2. Toxicology
3. Testing	3. Testing
4. RWO	4. RWO

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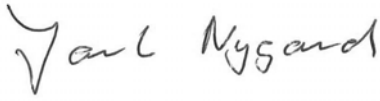

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Preface

The tests were conducted in 2008 at NIVAs test facility located at Solbergstrand 20 km south of Oslo. NIVA conducted the testing as a contract assignment for RWO, with The German Administration (BSH) as verifier.

During planning and conducting of the tests, Dr. Anja Kornmüller, Thomas Gerstmann and Martin Werkman were the lead representatives of RWO. From NIVA, Stephanie Delacroix, Anne-Marie Bomo, Oddbjørn Pettersen, Per Ivar Johannesen, August Tobiessen, Tor Gunnar Jantsch and Helge Liltved were the main representatives. Several other RWO and NIVA personell have been involved in the project.

We will use the opportunity to thank RWO for choosing NIVA as the main partner in the process of testing and verification of the CleanBallast ballast water management system, and thank all involved personell for the professionalism demonstrated in completing this project.

Oslo, December 2008

Tor Gunnar Jantsch

Contents

Abbreviations and acronyms

APHA – American Public Health Association
A. franciscana – Artemia franciscana
B. submarina – Brachiomonas submarina
AOX – Adsorbable organic halogens
BSH - The German Administration BSH (Federal Maritime and Hydrographic Agency)
CFDA-AM - 5-carboxyfluorescein diacetate acetoxymethyl ester
COD – chemical oxygen demand
CT2 – storage tank for control water (5 day)
DBP – disinfection byproducts
DNV – Det Norske Veritas
DO – dissolved oxygen
DOC – dissolved organic carbon
DQIs – data quality indicators
EC_{50}, EC_{10} - the concentrations causing 50 and 10 % effect, respectively, on the test organism
E. coli – Escherichia coli
EOX – Extractable organic halogens
EPA – Environmental Protection Agency (US)
FNU – Formazine Nephelometric Units
GC- gas chromatography
GF/F – glass fiber filter grade F
GLP – Good laboratory Practice
HAA- Haloacetic acids
IMO – International Maritime Organization
ISO – International Organisation for Standardization
LC_{50} – the concentration causing 50 % mortality of the test organism
LOQ- limit of quantification
MSD- mass spectrometry detection
n – number of measurements; in calculating the standard deviation
NDIR - Nondispersive Infrared
NIVA – Norwegian Institute for Water Research
NS-EN ISO – Norwegian, European and International Standard
OECD – Organisation for economic Co-operation and Development
PAR – photosynthetic active radiation
POC – particulate organic carbon
PSU – Practical Salinity Unit (= ‰)
QAPP – quality assurance project plan
QA/QC – quality assurance/quality control
QMP – quality management plan
S1-S5 – sampling points 1-5
SPE – solid phase exctraction
Std – standard deviation
TCBS - Thiosulphate citrate bile salt agar
THM – trihalomethanes
TOC- Total organic carbon
TRO- Total residual oxidants
TSS – total suspended solids
TT1 – (CT1) storage tank for treated water (5 day)/Tank for collection of deballasted control water
TT2 – Tank for collection of deballasted treated water
VOC- Volatile organic compounds
WST – tank with prepared test water

X_i - individual analytical result; in calculating the standard deviation
X – the arithmetic mean of individual analytical results; in calculating the standard deviation

Summary

Land-based testing of the CleanBallast ballast water management system of RWO has been completed in the period of September 2008 to November 2008. The testing has been conducted according to the IMOs *Guidelines for approval of ballast water management systems (G8), Res. MEPC 125(53) Annex 3* and *Procedure for approval of ballast water management systems that make use of active substances (G9) Res. MEPC 169(57) Annex 4*, hereafter referred to as G8 and G9. The tests were carried out at NIVA's test site located at Solbergstrand 20 km south of Oslo, with medium salinity and high salinity test water. A total of 13 test cycles have been completed. Each cycle lasted for a period of 5 days.

The test water was prepared in a common 516 m^3 tank (WST). A combination of indigenous harvested organisms and cultured surrogate species (>50 µm group: *Artemia fransiscana*; 10-50 µm group: *Tetraselmis suecica* and *Brachiomonas submarina*) were added to the test water. *Tetraselmis suecica* is approximately 10 µm in minimum dimension and is regarded as representative of the 10-50 µm group of organisms. This organism is considered to be a robust organism. In addition *Brachiomonas submarina* (approx. 12 µm in minimum dimension) and a natural collection of harvested organisms were added to fulfil the water quality requirement with regards to the 10-50 µm group. One test cycle included consecutive treatment of >200 m^3 test water by the CleanBallast BWMS (including the filter unit and the electrolytic unit), transferring the test water from tank with prepared test water (WST) to a ballast tank (TT1). The treated water was stored in TT1. After 5 days the water was pumped from TT1, treated in the electrolytic unit, to TT2 for sampling before discharge. The control cycle included transferring >200 m^3 of the same type of prepared test water from WST to a ballast tank (CT2) using the pump of the CleanBallast BWMS, but in by-pass of the treatment unit. Control water was stored in CT2 for five days. After the storage period, the control water was pumped to TT1 for sampling before discharge.

Fulfilment of the chemical and biological requirements of the test water

- The required levels for total suspended solids (TSS), dissolved organic carbon (DOC) and particulate organic carbon (POC) were fulfilled in all 13 test cycles.
- The requirements regarding influent density of the ≥50 µm group was met in all 13 test cycles. The requirements regarding the biological diversity within the population were also fulfilled in all tests.
- The requirements regarding influent density of the ≥10-50 µm group was met in all tests, for at least two out of three methods used for quantification.The requirements regarding the biological diversity within the population were fulfilled in all tests.
- The requirement regarding the concentration of heterotrophic bacteria in the influent water (≥10^4 CFU ml^{-1}) was fulfilled in all tests.

Biocidal effects of treatment and storage

- The required less than 10 viable organism ≥50 µm in minimum diameter per m^3 in the treated water after five days storage was fulfilled in 11 of 13 test cycles. In two tests (test cycle 1 and 2, after 5 days storage), the numbers of organisms failed to meet the requirement in samples analysed directly after treatment. When these samples were re-examined the next day, the numbers of alive artemia were below 10 per m^3. Investigations of possible contamination sources concluded that the reason for failure to meet the requirement was due to residual non-treated test water containing artemia in a segment of the outside piping of the test facility to the tanks. Better cleaning procedures and flushing of pipelines eliminated the problem in the remaining test cycles. Viability was determined by observation of movement. All samples were analysed immediately after samling and re-examinated after 24 hours. Re-examination of organisms ≥50 µm should be emphasized when assessing the efficiency of the technology as it

takes some time before full effect of the treatment is achieved. When re-examination the viability of organisms ≥50 µm after 24 hours, all 13 test cycles fulfilled the requirement (<10 organism /m^3). The requirement of non-treated control water stating that the level of viable organisms after 5 days of storage should be higher than 100 per m^3, was fulfilled in all 13 test cycles.

- The required less then 10 viable organisms ≥10-50 µm in minimum diameter per ml in the treated water after 5 days storage, as determined with three different detection methods, was fulfilled in all 13 test cycles. The equivalent requirement of non-treated control water stating that the level of viable organisms after 5 days of storage should be higher than 100 per ml, was fulfilled in all 13 test cycles.
- Regulation D-2 requires documentation of maximum allowable effluent concentrations after 5 days storage of *Eschericia coli*, *Vibrio cholera* (serotypes O1 and O139) and Intestinal *enterococci,* being <250 cfu/100 ml, <1 cfu/100 ml and <100 cfu/100 ml, respectively. These requirements were fulfilled in all 13 test cycles.

Total residual oxidants (TRO)
TRO was present in treated water immediately after deballasting in all test cycles. TRO measured as totale chlorine (mg/l Cl_2) was in the range from 0.76 – 1.47 mg/l Cl_2 for representative seawater tests, and in the range from 0.39 – 0.42 mg/l Cl_2 for brackish water tests. Test cycle 11 (seawater) was run with a neutralization step included in the deballasting process. TRO was not detected (<0.02 mg/l Cl_2) in treated water in this test cycle.

Disinfection byproducts (DBP)
In the present study, treated water in all test cycles were sampled and analysed for adsorbable organic halogens (AOX), extractable organic halogens (EOX), bromate, trihalomethanes (THMs) and other halogenated organic compounds. The dominating halogenated organic compound, and THM, was bromoform. After deballasting on day 5, bromoform was detected in concentrations in the range from 96-240 µg/l for brackish water and 86-200 µg/l for seawater. The dominating non organic byproduct was bromate, which was found in concentrations up to 29 µg/l in brackish water and up to 19 µg/l in seawater. The concentrations of the sum parameters AOX and EOX were surprisingly low compared to the concentrations of bromoform within the same test cycle.

Toxicity
The IMO regulations require that treated ballast water should be tested with regard to both acute toxicity and chronic effects using multiple test species (a fish, an invertebrate and a plant). Ballast water treated with the RWO CleanBallast treatment system, was tested with 6 different marine species covering 3 trophic levels and 5 phyla. A total of 40 toxicity tests have been performed. The results showed a remarkable uniformity with respect to degree of toxicity across species and phyla.

The algal tests gave the best picture of the variability in toxicity. There was a fairly good linear relationship with measured TRO in treated ballast water and observed toxicity. The algal tests showed that algae were equally or more sensitive to treated ballastwater than other test species. It is therefore relevant to use the algal test EC_{10} values for derivation of an aquatic PNEC in an environmental risk assessment.

The effect concentrations showed generally higher toxicity in test cycles with seawater than in test cycles with brackish water. In the seawater tests, the EC_{10} varied from 14 to 56 % (average 33 %) and EC_{50} from 19 to 78 % (average 44 %) when excluding test 11, day 5. In test cycle 11, day 5, the TRO was neutralized by addition of sodium thiosulphate and EC_{10} and EC_{50} were both >100%, showing that the neutralization step efficiently removed the toxicity. In all test cycles with seawater, the algae growth inhibition tests showed higher toxicity on day 5 than on day 0. The average EC_{50} values were

57 % on day 0 and 30 % on day 5. For brackish water the EC_{50} was always >100 % and EC_{10} was only occasionally less than 100 % on both day 0 and day 5.

All tests performed in brackish water showed a low toxicity of the treated ballast water. EC_{10} could only be calculated in four of the ten algae growth inhibition tests conducted. The treated ballast water gave less than 50% growth reduction in all tests and, hence, no EC_{50} values could be determined.

1. Background

The goal for RWO is certification of the RWO BWMS in accordance with the requirements in the IMO Convention on ballast water management (IMO, 2004) and the underlying guidelines; *Guidelines for approval of ballast water management systems (G8), MEPC 53/24/Add.1, Annex 3, Res. MEPC 125 (53), 2005* and *Procedure for approval of ballast water management systems that make use of active substances (G9) MEPC 57/21 Annex 1, Res. MEPC 169 (57), 2008,* section 1-5, pages 1-7. Guidelines are hereafter referred to as G8 and G9.

Land-based testing for type approval, as reported here, was conducted in the period of 3rd of September to 11th of November, 2008. The tests were conducted in accordance with G8 and G9.

2. Materials and test protocols

2.1 Test site

The tests were conducted at NIVA's test site located at Solbergstrand 20 km south of Oslo. Sea water was supplied from various depths down to 60 m in the Oslofjord, while fresh water was supplied from ground water bore holes or from a local creek.

The test facility includes 4 glass-fibre reinforced polyester tanks, supplied with inlet and outlet arrangements and equipment for proper cleaning. During storage of treated and control water, the tanks are covered to prevent light introduction (TT1, TT2 and CT2). The surfaces of the tanks are coated with coatings for ships (Balloxy HB light, Jotun, Norway). Propeller devices with gentle rotation are mounted at the bottom and at shallow depth in WST and TT1 (if necessary as judged by measurements of homogeneity), and at the bottom in TT2 and CT2. These propeller devices are used to suspend particles, including algae and zooplankton, evenly and homogenise the content. A measurement series is conducted to verify homogenisation prior to each test and prior to sampling.

2.2 The evaluated ballast water treatment system

2.2.1 General description of the RWO BWMS

In RWO's CleanBallast system the water is treated by using a mechanical separation step for sediment removal and an electrochemical treatment step for disinfection. For mechanical separation Disc Filters are used with a filter fineness of 50 µm. For disinfection the Active Substances are produced in-situ by the electrochemical treatment cell (EctoSys®) directly from the water without adding any chemicals. During ballast water uptake the CleanBallast system is operated inline using Disc filtration followed by the EctoSys® disinfection before the water enters the ballast water tanks. In general, re-growth of organisms is possible in the ballast water tanks during the voyage, which is simulated during the land-based type approval procedure by the five days tank storage. Therefore during deballasting, the Disc filter unit is bypassed and the EctoSys® disinfection is applied alone a second time before discharge.

At treatment during ballasting, a controlled limited current input from the rectifier to the EctoSys® cell assures that the Maximum Dosage disinfectant Concentration of 2 mg/L Total Residual Oxidant (TRO) is not exceeded in seawater. In fresh water (< 3 PSU) residual oxidants (Active Substances) are not detectable because the only formed Active Substance is the short-lived hydroxyl radical. At treatment during deballasting, the maximum input current applied is limited at all times ensuring the Maximum Dosage Concentration of < 2 mg/L TRO in marine waters. Furthermore, in waters of all salinities the applied current is mitigated by using the algae monitor to generate disinfectants only to the extent necessary at deballasting. By this, the TRO discharge concentration as well as the use of hydroxyl radicals is minimized in marine water qualities, and use of hydroxyl radicals is minimized in fresh water.

2.3 Test waters

Test water was prepared in a 516 m^3 tank (WST) from high salinity sea water from 60 meters depth or from brackish surface water depending on the required salinity, >32 PSU or 3-32 PSU (as specified in G8), respectively, with a minimum difference of 10 PSU. The 516 m^3 of test water was used for both testing and control. A combination of harvested indigenous organisms and cultured surrogate species (>50 µm: *Artemia franciscana*; 10-50 µm: *Tetraselmis suecica* and *Brachiomonas submarina*) were added to fulfil the biological water quality criteria, and soluble lignin, starch and kaolin was added to adjust the contents of dissolved organic carbon (DOC), particulate organic carbon (POC) and total

suspended solids (TSS), respectively, to within the limits of chemical water quality criteria as specified in **Table 1**.

2.3.1 Overview of requirements

Table 1 summarizes the chemical requirements of the test waters specified in G8, while **Table 2** summarizes the biological requirements.

Table 1. Required chemical water quality of test waters. Salinities should be separated by at least 10 PSU.

	Salinity	**DOC**	**POC**	**TSS**
Test water 1	>32 PSU	>1 mg/l	>1 mg/l	>1 mg/l
Test water 2	3-32 PSU	>5 mg/l	>5 mg/l	>50 mg/l

Table 2. Required biological water quality in influent test water, treated water and in control water after 5 days storage as stated in regulation G8 by IMO.

Organism group	**Influent water**	**In treated water after 5 days storage (Regulation D-2)**	**In control after 5 days storage****
≥50 µm min. dimension	Pref. 10^6 m^{-3}, $\geq 10^5$ m^{-3} Min. 5 species from 3 diff. phyla/divisions	<10 viable organisms per m^3	> 100 viable organisms per m^3
≥10-50 µm min. dimension	Pref.10^4 ml^{-1}, $\geq 10^3$ ml^{-1} Min. 5 species from 3 diff. phyla/divisions	<10 viable organisms per ml	> 100 viable organisms per ml
Heterotrophic bacteria	$\geq 10^4$ cfu ml^{-1}	-	-
Vibrio cholerae	-	<1 cfu/100 ml	-
Escherichia coli	-	<250 cfu/100 ml	-
Intestinal *Enterococci*	-	<100 cfu/100 ml	-

** As discussed in the results and discussion section.

2.3.2 Fulfilment of chemical water quality test criteria

Soluble lignin (40 % solution, Borrebond FP-P, Borregaard, Norway), starch (Maizena®, Unilever AS, Billingstad, Norway) and kaolin clay (VWR International, Oslo Norway) was added to adjust the contents of dissolved organic carbon (DOC), particulate organic carbon (POC) and total suspended solids (TSS), respectively, to within the limits of chemical water quality criteria as described in **Table 1**. The necessary additions of each compound were calculated on the basis of earlier laboratory tests and full scale experience. Prior to each test cycle , the content of TSS in the test water was checked by direct measurement on site, using the HACH method 8006 (HACH, 2001), applicable to samples with a TSS content within the range 0-750 mg/l, and the pre-set calibration standards of the Odyssey DR2500 spectrophotometer (HACH).

2.3.3 Fulfilment of biological water quality test criteria

A combination of indigenous harvested organisms and cultured surrogate species (>50 µm: *Artemia franciscana*; 10-50 µm: *Brachiomonas submarina* and/or *Tetraselmis suecica*) was added to fulfil the biological water quality criteria described in **Table 2**. Measurements by NIVA shows that *Tetraselmis suecica* has an average minimum diameter of 9.3 µm (n=25) when growing exponentially in our cultures, the diameter range is 7-11 µm. *Brachiomonas submarina* has a mean minimum diameter of 12.5 µm when growing exponentially with a range of 10-20 µm. *B.submarina* is a rather delicate organism with no protective outer shell. Even if it has the required minimum diameter it would not constitute much of a challenge for a ballast water treatment system. *T. suecica* on the other hand is

quite robust and has an outer shell composed of cellulose-like material. It has a good survival when exposed to shear forces in pumps and a good survival in the dark. In addition it is quite tolerant with respect to survival in brackish seawater. *T. suecica* is therefore a robust representative of the type of organisms to be expected in the 10-50 µm size fractions of marine organisms.

Cultivation of Artemia franciscana, Tetraselmis suecica and Brachiomonas submarina

A. franciscana: Resting cysts of *Artemia franciscana* are available commercially. Hatching of cysts are achieved by adding approximately 0.1 g of cysts to 1 litre of 20 ‰ salinity seawater. The culture is incubated with a bright light source at a temperature of 22-26 °C with good aeration. Full hatching is usually achieved within 48 hours. It is possible to hatch approximately 100 000 nauplii per litre. *Artemia* nauplii are hatched with a supply of food (egg yolk) and will therefore live for up to a week without any external food supply. Survival length is twice that if the naupli is stored at 8-10 ^{o}C. However, hatched nauplii should be used within 24 hours in order to achieve high survival and viability.

B. submarina and *T. suecica* were grown in seawater with an added commercial fertilizer (Superba, 0.3 g/l). Cultures were grown in plexiglass cylinders with a diameter of 30 cm and height of 180 cm. By following this procedure, densities of 1-3x10^9 cells of *T. suecica* per litre has been achieved.

The required cultivation volume was calculated based on a final density of 10^5 nauplii per m^3 for *A. franciscana* and 10^3 per ml for *B. submarina* and *T. suecica* assuming a final volume of the test water of 516 m^3.

Harvesting of indigenous organisms

Fulfilment of the criteria regarding at least five species from three different phyla/divisions of both test groups of organisms ≥10 µm in minimum dimensions was assured by harvesting indigenous algae and planktonic animal species from surface water outside Solbergstrand research station using a Unik Filter type 450 (Unik Filtersystems, Os, Norway) equipped with a 20 µm mesh size screen. The harvesting process has previously been shown to be relatively gentle to the organisms; surface water (1 m depth) was smoothly pumped (ca. 3 m^3/h) by a jet-pump to the inlet side of the screen, and algae and animals were washed from the screen into a collecting tray (ca. 100 l/h which gives approximately 30x concentration) and further to a storage tank. Water from the storage tank was transported through a pipe to WST by gravity. The species composition and viability of harvested organisms were evaluated by microscopy.

Bacteria

The concentration of heterotrophic bacteria in the surface water is normally exceeding the required level of ≥10^4 cfu ml^{-1}, while the heterotrophic bacteria criteria for the high salinity test water (> 32 PSU) is expected to be fulfilled by the heterotrophic communities accompanying the cultured *Tetraselmis suecica, Brachiomonas submarina* and *Artemia franciscana*. During the tests, it was not necessary to supply any extra bacteria added as cultivated heterotrophic bacteria.

2.4 Test procedure

2.4.1 Description of test cycle

The different water transfers between tanks via the RWO BWMS during a test cycle including the control is shown in **Figure 1**. One test cycle (blue lines) involved treatment of >200 m^3 test water by the RWO BMWS, during transfer of the test water from the tank with prepared test water (WST) to a ballast tank (TT1). The treated water was stored in TT1 for 5 days. After 5 days the water was pumped

to TT2 for sampling before discharge. Note that during transfer of water from TT1 to TT2 at day 5, the water was by-passed the filter but treated in the electrolytic unit (EctoSys®) of the BWMS.

A control cycle (red lines) was run by transferring >200 m^3 of the same type of prepared test water from WST to a ballast tank (CT2) for 5 days storage using the pump of the RWO BWMS, but in by-pass of the treatment unit. After storage the control water was pumped to TT1 for sampling before discharge.

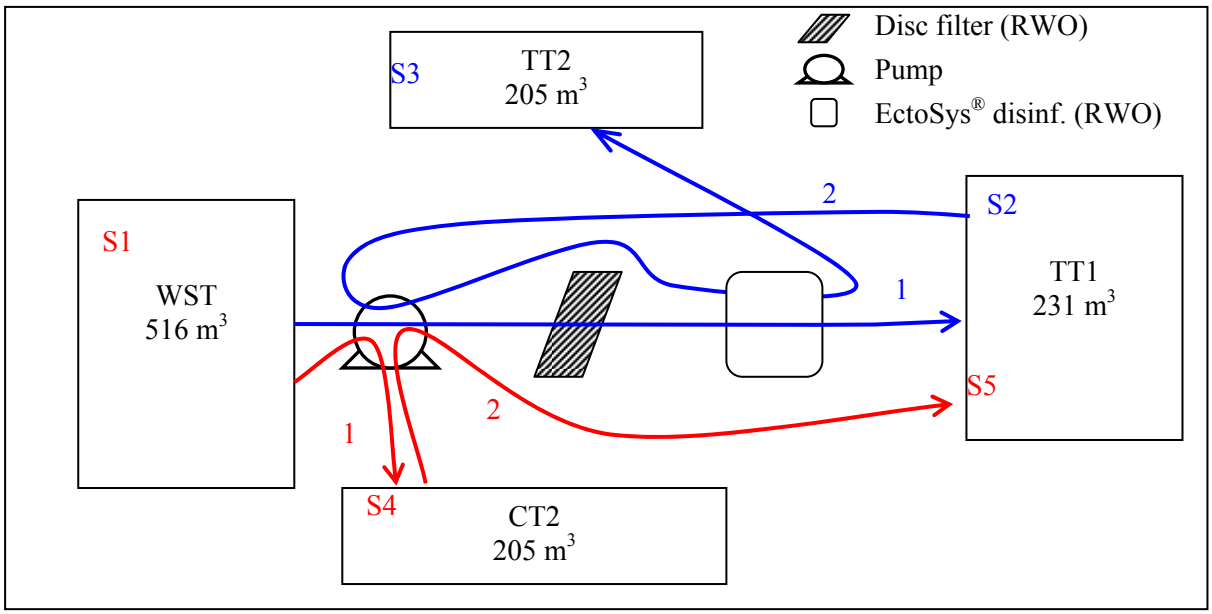

Figure 1. Transfer of test water during one test cycle with the CleanBallast BWMS including a test line (blue) and a control line (red). Blue line 1 indicates the day 0 ballasting operation of treated water, whilst blue line 2 indicates the day 5 deballasting operation of treated water. Red line 1 indicates the day 0 ballasting operation of control water. Red line 2 indicates the day 5 deballasting operation of control water. Sampling numbers are indicated by S1-S5.

2.4.2 Measures to avoid cross-contamination during water transfer and sampling

To avoid cross-contamination between consecutive test waters upon transfer between tanks, all pipelines and tanks were flushed for 2-3 min with sea water from 60 meters depth or ground water with documented quality between each test cycle, followed by rinsing with high temperature water (80-90°C).

The same holding tank (TT1) was used for both treated water and control water in the same test cycle. To avoid contamination, treated water was always introduced to TT1 before control water. No cross-contamination was therefore likely to occur because the density of organisms was lower in treated water than in control water.

To avoid cross-contamination during sampling, the buckets, siphon overflow and plankton net were rinsed in sea water from 60 meters between each sampling.

2.5 Sampling

2.5.1 Assuring the representativeness of samples

Before any samples were collected from any of the tanks at every sampling time the following procedure was carried out to assure that representative samples were withdrawn:

1) The particulate content of the 516 m^3 tank (WST) was homogenized using an outboard propeller of 3 hp and an electric mixer (Brio model 1.0, 0.75 kW). The outboard propeller was mounted on the edge of the tank, while the electric mixer was mounted at the bottom of the tank. The outboard propeller could mix alternately between the periphery and centre of the tank. Mixers (Brio Model 1.0, 0.75kW) were also mounted in TT1, TT2 and CT2 to provide homogenity before sampling.
2) The turbidity in different sections of each tank (upper part, middle part and bottom part, in both periphery and mid section) was measured by a handheld submersible probe (YSI – 600 OMS) during homogenization. When any turbidity measurement was within a 10 % deviation from the average turbidity of all measurements in the tank, sampling was started.

2.5.2 Sampling protocols

The following procedures were used to collect samples from the different tanks and sampling times. All sampling were done in triplicates.

1) *Sampling of bacteria in WST (S1), TT1 (S2), TT2 (S3), CT2 (S4) and TT1 (S5)*: Bacterial samples were collected as 3x 1000 ml grab samples by slowly submerging a 1000-ml sterile bottle. The bottle was closed immediately after sampling. Bottles for bacterial sampling contained thiosulphate for preservation purposes.
2) *Sampling of organisms ≥ 50 µm in WST (S1), CT2 (S4) and TT1 (S5).* A plastic bucket was used to collect 3x 20-100 litre sample. The sampled water was slowly sieved through a plankton net (50 µm diagonal dimensions) attached to a plastic cup, collecting the organisms in the cup.
3) *Sampling of organisms ≥ 50 µm in TT1(S2) and TT2 (S3):* A siphon spillway was used to collect 3x 1 m^3 test water from TT1 directly after treatment, and from TT2 after 5 days storage. The water was sieved directly through a plankton net (50 µm diagonal dimensions) attached to a plastic cup, collecting the organisms in the cup. The sieved water was collected in known volume tanks to ensure accurate sampling volume.
4) *Sampling of organisms 10-50 µm in WST (S1), TT1 (S2), TT2 (S3), CT2 (S4) and TT1 (S5)*: Organisms with a minimum diameter between 10 µm and 50 µm were sampled as 3x 1000 ml for control water with clean glass bottle and 3x 10 l for treated water with a clean plastic bucket.
5) *Sampling of test water for pH, salinity, organic carbon measurements in WST (S1), TT1 (S2), TT2 (S3), CT2 (S4) and TT1 (S5)*: Water samples were collected as 3x 1000 ml grab samples by slowly submerging a 1000-ml clean plastic bottle. The bottle was closed immediately after sampling.
6) *Sampling of test water for TRO measurements (including decay test):* Water samples were collected as 3x 1000 ml grab samples by slowly submerging a 1000-ml clean glass bottle pretreated with bleach to remove any chlorine demanding substances. TRO was measured immediately after sampling.
7) *Sampling of test water for DBP measurement (including decay test):* Water samples were collected as 6x 1000 ml grab samples by slowly submerging a 1000-ml ALS glass bottle. The bottle was top-filled and closed immediately after sampling and stored at 4°C. Glass bottles for DBP sampling contained 100 mg thiosulphate for preservation of samples.
8) *Sampling of test water for acute, chronic and sub-chronic toxicity test:* Water samples were directly collected as 1x 1000 ml grab samples by slowly submerging a 1000-ml clean glass bottle. The bottle was closed immediately after sampling. For fish toxicity test, more than 300 l test water was directly collected in stainless steel containers. The test water was acclimatised to test temperature prior to exposing the fish to the test water.

9) *Sludge samples:* Sludge from the filter back wash water was sampled twice during the testing programme.

2.5.3 Overview of sampling equipment

An overview of sampling equipment, containers used and sampled volumes are given in **Table 3**.

Table 3. Equipment and containers used for sampling and necessary sample volume for the individual parameters.

Parameter	Sampling equipment	Sample container	Collected volume WST, CT2	Collected volume TT1, TT2
Turbidity	Turbidimeter submersible probe	-	-	-
pH	pH probe	-	-	-
Temperature	Temp. meter	-	-	-
Salinity	Probe			
Dissolved oxygen	DO probe	-	-	-
Redox	Probe	-	-	-
DOC*	Directly			
POC*	Directly			
TSS	Directly	Clean plastic bottle	1000 ml	1000 ml
Disinfection by-products and chemical fate analysis (DBP)	Directly	ALS laboratory glass bottle with 100 mg/l thiosulphate	1000 ml	1000 ml
Total residual oxidants (TRO)	Directly	Clean glass bottle pretreated with bleach to remove any chlorine demanding substances	2000ml	2000ml
Sludge characterization	Directly from filter backwash	Clean plastic bottle	1000 ml	1000 ml
Organisms ≥ 50 µm	Sieving**	Clean glass bottle		
Organisms 10-50 µm	Directly	Clean glass bottle, clean plastic bucket		
Heterotrophic bacteria				
Coliform bacteria, *E. coli*				
Enterococcus group bacteria	Directly	Sterile bottle with thiosulfate	1000 ml	1000 ml
Vibrio sp.				
Vibrio cholerae				
Acute/Chronic Algae, Copepods, rotatoria, oyster embryo	Directly	Clean glass bottle	1000ml	1000ml
Acute fish, Juvenile fish	Directly	Stainless steel container	300l	300l

* According to the concervation procedures at the laboratory, samples from the 1000 ml bottle are transferred to 100 ml acid washed glass bottles.

** A 20-100-litre grab sample (from WST and CT2) or 1 m^3 collected through a siphon spillway (from TT1, TT2) was concentrated to a volume of 40-100 ml through plankton net with diagonal dimensions of 50 µm.

2.5.4 Sample preservation and transportation

Preservation methods and expected storage/holding times before measurement are shown in **Table 4**. All preservation and analysis were in accordance with the described methodology. After sampling, samples were placed in the dark in cooling-containers and transported to the laboratories for analysis.

Table 4. Preservation methods and expected storage/holding times before measurement (ISO/CD 5667-3, 2001).

Parameter	Preservation	Maximum holding time	Expected storage time
Temperature	-	-	0
pH	-	-	0
Dissolved oxygen	-	-	0
Salinity	-	-	0
Turbidity	-	-	-
Redox	-	-	0
TRO	-	-	0
Disinfection by-products (DBP)	Chlorine neutralisation with thiosulphate. Stored in dark, 4°C, top-filled bottles	5 days	0-5 days
Dissolved organic carbon (DOC)	Acidify with 1 ml 4 M H_2SO_4 per 100 ml (pH<2), 4°C	7 days	0-5 days
Total organic carbon (TOC)	Acidify with 1 ml 4 M H_2SO_4 per 100 ml(pH<2), 4°C	7 days	0-5 days
Particulate organic carbon (POC)	Acidify with 1 ml 4 M H_2SO_4 per 100 ml(pH<2), 4°C	7 days	0-5 days
Total suspended solids (TSS)	4°C	24 hours	<24 hours
Organisms ≥ 50 µm	4°C	6 hours	< 2 h
Organisms 10-50 µm	4°C	24 hours	< 24 h
Heterotrophic bacteria	4°C	24 hours	< 24 h
Coliform bacteria, *E. coli*			
Enterococcus group bacteria			
Vibrio sp.			
Vibrio cholerae			
Acute/Chronic Algae, Copepods, rotatoria, oyster embryo	4°C	24 hours	< 24 h
Acute fish, Juvenile fish	16°C	24 hours	< 24 h

2.6 Analyses

2.6.1 In situ measurements

Temperature

Temperature was measured *in situ* using a calibrated thermometer. Temperature is reported in ºC.

pH

pH was measured *in situ* using a calibrated probe and pH-meter.

Dissolved oxygen (DO)

Dissolved oxygen (DO) was measured *in situ* using a calibrated probe and meter. DO is reported as mg O_2/l.

Salinity

Salinity was measured *in situ* using a calibrated salioterm. Salinity is reported in PSU or in ‰.

Redox potential

Redox potential was measured *in situ* using a Walchem Wbh320-5nn controller with a pH/ORP Thermo Orion electrode. Redox potential is reported in mV.

2.6.2 Discrete samples

Total residual oxidants (TRO)

Total residual oxidants were measured by the colorimetric DPD-method which is currently the method recommended for measurement of TRO in seawater (APHA, 2005). The method is based on the oxidation of N,N-diethyl-p-phenylendiamin (DPD) which turns to a pink Wurster-cation in the presence of strong oxidants. The intensity of the colour is proportional to the TRO concentration. The colour intensity was measured by a Hach DR/2000 spectrophotometer (Hach Company, Loveland, CO, USA). The method and the instrument give the results as total residual oxidants (TRO) in mg/l Cl_2, and TRO was measured both as free chlorine (Hach method 8021) and total chlorine (Hach method 8167). Sample water without reagent was used as blank. Concentration of TRO was measured daily in the storage tanks for treated water and control water. In addition, a decay study of TRO was performed after deballasting for each test cycle.

Dissolved and total organic carbon (DOC and TOC)

DOC and TOC was measured by an accredited method based on Norwegian Standard NS-ISO 8245 (NIVA method G5-3) at NIVA. TOC is measured on the whole sample and DOC is measured after filtering the sample through a GF/F filter (0.7 µm). The sample was acidified with phosphoric acid and aerated with oxygen to remove inorganic carbon prior to injection into a quartz tube filled with a platinum catalyser at 680 ^{o}C. The organic carbon compounds were oxidized to CO_2 which was quantified using a NDIR detector (Phoenix 8000 TOC-TC analyser with sample carousel STS 8000) with oxygen as carrier. Detection limit was 0.2 mg C/l.

Particulate organic carbon (POC)

POC is calculated as the difference between the level of total organic carbon in the sample, measured on the non-filtered sample (see above), and the measured DOC level of the same sample. POC was also measured at NIVA (method G6) as the amount of organic matter accumulating on a glassfiber filter GF/F (0.7 µm) when a known amount of sample is filtered. The dry sample is encapsulated in tin capsules which are ignited in oxygen supersaturated helium gas at 1800 ºC. Surplus oxygen is removed by Cu at 650 ºC and the off-gases are passed through a chromatographic coloumn, where CO_2 is detected (Carlo Erba Elementanalysator 1106 whith sample changer AS 400 LS). The method is based on Carlo Erba Instrumentazione Eelemental Analyser 1106. Instruction Manual Application lab reports, Elemental Analysis Lab, Carlo Erba, 1987. Detection limit is 1.0 µg C/l.

Total suspended solids (TSS) and ignition loss

TSS was measured at NIVA (method B1/2) in accordance to NS-EN 872 and NS 4733. A glassfiber filter GF/F (0.7 µm) was washed with distilled water, dried at 105 ^{o}C for 30 minutes, then ignited at

480 ºC for 2 hours and finally weighed. The sample was filtered through the filter prepared as described above, and filtered samples are dried for one hour and weighed. The TSS represents the weigh increase. Lowest reported value is 0.1 mg/l. The filter with the residue was then ignited at 480 ºC and the ignition loss determined by weighing. The ignition loss is showed in percentage (%) of the ignition residue of the sample.

Density

Density was determined at NIVA as the weight of a known volume of the sample, divided by the volume of the sample.

Disinfection by-products

Disinfection by-products were sampled and immediately shipped to subcontractors (ALS Scandinavia and DVGW - Technologiezentrum Wasser (TZW) for analysis (within 5 days). In accordance with documents MEPC 57/2/3 and GESAMP –BWWG 4/9, samples were analysed for all relevant disinfection by-products in each test cycle. In addition, two chemical fate studies were done – one for seawater (test cycle 2) and one for brackish water (test cycle 9).

AOX

Determination of adsorbable organically bound halogens (AOX) is described in DIN EN ISO 9562:2004 by a solid phase extraction (SPE) in waters with high salt content. AOX represents the sum of organically bound chlorine, bromine and iodine (but not fluorine) which can be adsorbed on activated carbon under specified conditions and, if the sample is not filtered, includes that associated with suspended matter.

EOX

Extractable organically bound halogens (EOX) were measured by hexan extraction with microcolorimetric tittering as described in DIN 38409-H8:1984.

THM

DIN EN ISO 10301-F4:1997 describes a general method for the determination of highly volatile halogenated hydrocarbons in water by gas chromatography (GC). The detection was carried out by mass spectrometry.

Bromate

Due to the interference by the chloride concentration in brackish water and full salinity seawater, which creates major interference problems in the standard method, customized laboratory method using IC-ICP-MS technique was applied for the analysis of bromate with a detection limit of 1 µg/L.

Haloacetic acids

Haloacetic acids (HAA) was determined by gas chromatography as it is described in ISO 23631 (2006)- Water quality — Determination of dalapon, trichloroacetic acid and selected haloacetic acids - Method using gas chromatography (GC-ECD and/or GC-MS detection) after liquid-liquid extraction and derivatization.

Bromoacetonitrile

Bromoacetonitrile compounds could not be analysed.

Bromophenol

Bromophenol compounds were measured by gas chromatography with mass spectrometry detection as described in DIN EN 12673:1999. Gas chromatographic determination of some selected chlorophenols in water.

Other volatile organic compounds
Volatile organic compounds were purged from the sample matrix into a gaseous phase at the inlet stage of a gas chromatography mass spectrometer. The inlet was then heated and back flushed with inert gas to desorb the compounds onto the gas chromatographic column. The gas chromatograph was temperature programmed to separate the volatile compounds and detection was by mass spectrometry.

Sludge characterization

In one test cycle for each salinity range the backwash water from the filter was analysed for sludge characterization to be compared to the influent water at ballasting. The analysis included the following parameters:

- pH-value (as described above)
- Salinity as practical salinity units (as described above)
- Dissolved Oxygen (on-site) (as described above)
- Redox potential (as described above)
- Total Suspended Solids (TSS) (as described above)
- Total Organic Carbon (TOC) (as described above)
- Dissolved Organic Carbon (DOC) (as described above)
- Particular Organic Carbon (POC) (as described above)
- Total dry matter (TTS) (as described above)
- Ignition loss (as described above)
- Density (as described above)
- Turbidity (as described above)

Determination and quantification of organisms ≥50 µm

Organisms ≥50 µm were inspected in microscope at 10-40x magnification within 6 hours after sampling. Viable organisms were counted and identified based on motility and integrity according to OECD (1985): OECD Test Guideline for Testing of Chemicals 202, "*Daphnia* sp. acute immobilisation test and reproduction test".

Determination and quantification of organisms ≥10-50 µm

The viability of the micro-plankton (≥10-50 µm) was determined by observing cells incubated with 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM) according to Ganassin et al. (2000). A 10 ml sample was incubated for 1 hour with 4 µmol of CFDA-AM. The sample was fixed with formalin and filtered onto black polycarbonate filters (25 mm). The filter was mounted on a glass slide in paraffin oil and frozen. CFDA-AM is hydrolysed only in a living cell. CFDA-AM is a marker for cell membrane integrity and may be measured directly in cells. In principle, the non-fluorescent chemicals CFDA-AM is taken up in the cytosol, where it becomes hydrolysed into fluorescence end products. These end products are trapped inside the cellular compartment and may be observed in an epifluorescence microscope using excitation filter 485 nm and emission filter of 530 nm. In the epifluorescence microscope viable cells are observable as brightly yellow/green coloured cells, while non viable cells are pale green (heterotrophic cells) or pale green with red autofluorescence of the chloroplast (photoautotrophs). Numbers of viable and non viable cells were counted at 300x – 480x magnification.

As a complementary method to direct counting for testing of viability, the serial dilution method in algal growth medium was used. The serial dilution method is often referred to as the most probable number method. It is simply based on the fact that by diluting the sample in a sequence and observing in which dilutions the organisms occur (grow) in afterwards, one is able to backward calculate the number of cells in the original sample. The dilution series were achieved by adding 1 ml of sample to

9 ml of media (algal growth media, 20 % Z8 seawater media). After mixing, 1 ml of this sample was further diluted with 9 ml. In this way a series of 10x dilution were made. The number of dilutions was set to cover the expected cell density range in the original sample. 3-5 parallels were employed in order to provide statistical significance of estimated number.

A supplementary cultivation test was also used by plating on agar plates. 100 µl of samples was spread out on a seawater agar growth medium and incubated in constant light for 72 hours at 20 ^{o}C. Colonies of *Tetraselmis sp.* was observed by viewing agar plates in stereo microscope at 160x magnification. The procedure has a detection limit of 10 cells/ml, and was used as a rapid estimation of viable *Tetraselmis sp* in the samples.

Bacteria

Generally the samples were diluted or concentrated to achieve a quantifiable concentration of colony forming units on a solid growth media (agar-medium plates) or a medium-amended filterpillow. The dilution or concentration was based on experience and expectance of the concentration of the bacteria in the sample. Dilution was performed by stepwise 10x dilution of the sample in a dilution series followed by incubation on agar. Concentration was performed by filtering a predetermined sample volume through a sterile filter followed by incubation of the filter on growth media.

Heterotrophic bacteria
Heterotrophic bacteria were quantified according to a modified version of Norwegian Standard NS-EN 6222:1999 using a marine agar for isolation of marine heterotrophic bacteria.

Coliforms
Coliform bacteria were quantified according to Norwegian Standard NS 4788 at a temperature of 37±1°C and an incubation period of 22-24 hours.

E.coli
E. coli were quantified according to Norwegian Standard NS 4792 or NS-EN ISO 9308-3 at a temperature of 44.5±0.2 °C and an incubation period of 18-24 hours.

Enterococcus group
Total fecal *Enterococci* were quantified according to Norwegian Standard NS-EN ISO 7899-2 at a temperature of 36±2 °C and an incubation period of 44 hours.

Intestinal *Enterococci*
Intestinal *Enterococci* were confirmed according to Norwegian Standard NS-EN ISO 7899-2 at a temperature of 44±0.2 °C and an incubation period of 2 hours.

Vibrio species *and Vibrio cholerae*
The total number of *Vibrio* sp., were determined by filtration of 1-100 ml sample, and by placing the filter on TCBS Cholera-medium agar plates (CMO333 from Oxoid). Plates were incubated at 37 °C, and colonies counted after 24 hours incubation. The TCBS Cholera-medium supports the growth of pathogenic Vibrios (e. g. *Vibrio cholerae*, *Vibrio parahaemolyticus*) as well as some other Vibrios and other bacterial species, i.e. *Aeromonas hydrophila.*

The strategy for elimination or identification of serotypes O1 and O139 were as follows:
The morphology of the colonies developing on the TCBS-medium after 24 h was visually studied. Colonies with distinct colour and morphology different from *Vibrio cholera* were not selected for further identification. Colonies with typical *Vibrio cholera* appearance were re-striked on TCBS medium and again inspected for growth and morphology. Classical elimination or identification methods were used, such as appearance in culture media and physiological and biochemical

properties. If *Vibrio cholera* was identified, polymerase chain reaction (PCR) would be used for elimination or identification of the serotypes O1 and O139.

2.6.3 Toxicity measurements of treated ballast water

The following standard tests were performed on the treated ballast water.

- Growth inhibition of the marine alga *Skeletonema costatum* according to ISO 10253: Marine algal growth inhibition test.
- Acute toxicity to the marine crustacean *Acartia tonsa* according to ISO 14669: Determination of acute lethal toxicity to marine copepods (*Copepoda, Crustacea*).
- Reproductive toxicity to the marine crustacean *Nitocra spinipes* according to "Forslag til Dansk Standard: Økotoksikologisk undersøgelse med krepsdyret *Nitocra spinipes*. Reproduksjonsmetode".
- The oyster embryo bioassay according to the ASTM method (E724) and the comprehensive guidelines laid out in the ecotoxicity test methods for effluent and receiving water assessment (EA, 2001).
- Acute toxicity towards the juvenile turbot (*Scopthalmus maximus*) according to OECD Guidelines for testing of chemicals (No. 203; Fish, acute toxicity test).
- Chronic toxicity towards the juvenile turbot (*Scopthalmus maximus*) according to OECD Guidelines for testing of chemicals" (No. 215; Fish, juvenile growth test), adapted for marine species.
- Chronic toxicity using rotatoria reproduction test with the marine species *Brachionus plicatilis* based on a standard test developed for the related freshwater species *Brachionus calyciflorus* (ISO/FDIS 20666 – Determination of the chronic toxicity to *Brachionus calyciflorus*).

Sampling of treated ballastwater for toxicity tests with algae and invertebrates

Test water for these tests was taken in connection with the general sampling routine of day 0 or day 5. All samples were grab samples transferred into 2 liter glass bottles (Sorvall). The bottles were transported to the laboratory in cooling bags within 2 hours of sampling. Upon arrival at the laboratory the samples were put in a cooling room with a temperature of 4 ^{o}C. In general the test water was filtered through GF/C filter before being used in the tests. Further treatment of test water is described in the test reports (**Appendix K – Toxicity tests**).

Sampling of treated ballastwater for toxicity tests with fish

Sampling of test water was undertaken directly after finishing the general sampling routine on day 5. A hose was connected to the gravity sampling hose and the test water was led directly to several 300 L stainless steel storage tanks in a climate room were the fish tests were undertaken. This allowed the water to acclimatise to the test temperature prior to use. Fish were exposed to the testwater without any other pre-treatment.

Growth inhibition of the marine alga *Skeletonema costatum*

The inhibitory effect of treated ballast water on the growth of the marine diatom *Skeletonema costatum*, strain NIVA BAC1, has been investigated. The test was performed according to ISO 10253: Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum.*

A concentration series of treated ballast water diluted in untreated water was prepared. The batches were inoculated with test algae and incubated on a shaking table at 20 ± 2 °C, under continuous illumination. Growth was monitored by daily counting of cell numbers using a Coulter Multisizer for three days. The tests were performed with three replicates at each concentration and six control replicates in untreated ballast water.

The growth rate in each culture was calculated from the increase in cell density during three days exposure. Growth rates were calculated as percentage of growth rate in the controls (untreated ballast water) and plotted against concentration of treated ballast water. From the respons plot, the concentrations causing 10% and 50 % inhibition of the growth rate (i.e. EC_{10} and EC_{50}) were derived by non-linear regression analysis.

Reproductive toxicity to the marine crustacean *Nitocra spinipes*
The reproductive toxicity of treated ballast water to the marine crustacean *Nitocra spinipes* has been investigated. The test was performed according to Draft guideline for Danish Standard: "Økotoksikologisk undersøgelse med krepsdyret *Nitocra spinipes*. Reproduksjonsmetode". The test was performed as a limit test with 100 % treated ballast water and using non treated ballast water as control water.

The test was performed with 20 replicate vessels with 1 pregnant female in each vessel. The vessels were incubated for 14 days at 20 °C. The total number of living offspring was counted.

Acute toxicity to the marine crustacean *Acartia tonsa*
The acute toxicity of treated ballast water to the marine crustacean *Acartia tonsa* has been investigated. The test was performed according to ISO 14669: Determination of acute lethal toxicity to marine copepods (*Copepoda, Crustacea*). The test concentrations were in the range 32 to 100 % of treated ballast water.

The test was performed with four replicate vessels with 4-8 test animals for each test concentration and sixteen control replicate vessels. The vessels were incubated for 48 hours at 20 ± 1 °C. Mortality was recorded after 24 and 48 hours.

Chronic toxicity towards the juvenile turbot (*Scopthalmus maximus*)
The chronic toxicity of treated ballast water towards turbot was tested in accordance with the "OECD Guidelines for testing of chemicals" (No. 215; Fish, juvenile growth test), adapted for marine fish (McWilliams, 1994)

The testing of chronic toxicity of treated ballast water is required by the IMO G9 guidelines for testing of treatment technology to be used onboard ships. The fish was exposed continuously for 28 days with water exchange 3 times per week.

The test water was taken directly from control and test tank (CT1 and TT2) (100% ballast water), after conditioning to the test temperature.

Acute toxicity towards the juvenile turbot (*Scopthalmus maximus*)
The acute toxicity of treated ballast water towards turbot was tested in accordance with the draft procedure of McWilliams (1994). The procedure follows the general guidelines of OECD 203 "Fish, Acute toxicity test".

The testing of acute toxicity of treated ballast water is required by the IMO G9 guidelines for testing of treatment technology to be used onboard ships. The fish was exposed continuously for 96 hours with full water exchange every day.

10 juvenile turbot were used in each aquarium with 40 l of medium. The test water was taken directly from the control and test tank (CT1 and TT2) (100% ballast water), after conditioning to test temperature.

The oyster embryo bioassay
The oyster embryo bioassay (OEB) is based on the ASTM method (E724) and the comprehensive guidelines laid out in the ecotoxicity test methods for effluent and receiving water assessment (EA,

2001). The OEB is a sensitive in vivo test that measures the response of the most sensitive life stage of the oyster to contaminant exposure. The bioassay measures the success of trocophore larvae to develop into a normal D-stage veliger larvae following 48 hour exposure to test media. The frequency of normal D-stage larvae following 48 hour exposure is determined microscopically to provide an assessment of sample toxicity. The data generated enables standard toxicity values such EC_{10}, EC_{50} and NOEC (no observable effect concentration) and LOEC (lowest observable effects concentration) values to be determined for the test sample.

Rotatoria reproduction test
The chronic toxicity to rotatoria was studied using the marine species *Brachionus plicatilis*. The rotifera were kept in a laboratory culture fed with live algae. The test procedure is based on a standard test developed for the related freshwater species *Brachionus calyciflorus* (ISO/FDIS 20666 – Determination of the chronic toxicity to *Brachionus calyciflorus*). Briefly, freshly hatched rotatoria were incubated individually in a series of concentrations of the test water and in control water for 72 hours. At the end of the test, the number of egg and offspring were determined and compared with the control, i.e. the non treated ballast water. The population growth percentages were determined for each concentration of the test water.

2.6.4 Hydrogen and oxygen gas measurements

In the CleanBallast system the gas (hydrogen and oxygen), which might be produced by the EctoSys® is automatically removed by a gas trap after the EctoSys® and further by a deaeration pipe to the ambient environment via an air release valve working continuously at the top. In the trapped gas the concentration of oxygen was measured by the gas analyser Dräger Polytron 7000 (sensor 6809720, works calibration 0 - 25 Vol-% O_2) and of hydrogen Dräger PEX 3000, Typ XTR 0091 (sensor SE Ex PRM, works calibration 0 - 4 Vol-% H_2) installed in the exhaust gas line. The oxygen and hydrogen gas concentrations were automatically logged and stored in the panel PC of the CleanBallast system. Additionally, in some of the test cycles the overall gas volume leaving the deaeration pipe was trapped and the volume was recorded under the supervision of personell from NIVA. Manual measurements of hydrogen gas concentrations were also carried out with a portable analyser (Dräger PACIII) at different times (start, middle and end) in the storage tanks during ballasting and deballasting.

3. Results and discussion

3.1 QA/QC procedures

Quality assurance and quality control have been performed during the testing according to Chapter 5 in the QAPP and according to G8. All activities and data collected during testing of the RWO treatment system have been logged as summarized in **Table 5**. For each activity a specially designed log in paper and/or electronic format has been used (**Appendix A – K, O and P**). These log sheets were also used as quality assurance of some of the operations perfomed during the tests. For appendix L, M and N please refer to the QAPP made for this project.

Table 5. Log protocols for all activities in the project.

Appendix	Description
A	Total project management
B	Chemical water quality preparation/Homogeneity
C	Biological water quality preparation
D	Operational data
E	Sample collection
F	Sample handling
G	Logging of *in situ* measurements
H	Evaluation form for organisms >50 µm
I	Evaluation form for organisms ≥10-50 µm
J	Evaluation form for heterotrophic bacteria, coliforms, *E. coli*, Enterococcus group, intestinal *Enterococci*, Vibrio spp. and *Vibrio cholerae.*
K	Toxicity tests
L	Process description (described in QAPP)
M	System start up procedures (described in QAPP)
N	Laboratory sub contractors (described in QAPP)
O	Disinfection by-products methods, test reports from sub-contractors
P	Measurements of free and total chlorine (TRO)

3.2 Operational performance of the RWO BMWS

A total of 13 test cycles have been completed in the period of 3th September to 11th November, 2008. Test cycles 1-5 and 11-13 were conducted with high salinity water (>32 PSU) and the remaining with medium salinity (brackish water) (< 22 PSU). In test 11 an additional neutralisation system (dosing of sodium thiosulphate) was used after the CleanBallast system during deballasting to eliminate remaining TRO. Due to problems with residue artemia in the treatment system pipeline, too high numbers of artemia was recorded in treated water on day 5 for two of the seawater tests (test cycle 1 and 2) (as discussed in section 3.6). In addition, problems with the TRO online measurement, which monitors the concentration of Active Substances produced by the EctoSys® system, occurred in test cycle 1, which caused too high TRO concentration at deballasting as well as toxic water. Due to the contamination and as test cycle 1 and partly test cycle 2 deviates from a normal and satisfying operation of the CleanBallast treatment system it was decided to run two extra seawater tests (test cycle 12 and 13) in order to achieve five test cycles within one salinity range that fulfills the IMO requirements and shows representative values for TRO and toxicity.

Each test cycle lasted for 5 days. The dates of the cycles and the time for start/stop of each step in the test cycle are given in **Table 6**.

A control of the operation performance of the technology was performed in each test cycle by the site responsible person from NIVA. Reports from these controls are included in **Appendix D**. Check points and operational parameters for documenting sufficient operation of the treatment technology were included and monitored in this quality control, including start and stop of ballasting and deballasting of test water, and ballasting and deballasting of control water.

Table 6. Test cycles completed with dates and the time for start/stop of each step in the cycle.

Test cycle	Date	Start/stop ballasting WST to TT1 Day 0	Start/stop deballasting TT1 to TT2 Day 5	Start/stop control ballasting WST to CT2 Day 0	Start/stop control deballasting CT2 to TT1 Day 5	Salinity PSU
1	3th – 8th Sep. 08	20:13 – 20:38	12:58 – 13:22	21:03 – 21:27	14:05 – 14:28	>32
2	10th – 15th Sep. 08	11:22 – 11:57	11:27 – 11:51	12:18 – 12:43	12:32 – 12:54	>32
3	17th – 22th Sep. 08	11:20 – 11:46	09:35 – 09:55	11:59 – 12:23	10:26 – 10:47	> 32
4	22th – 27th Sep 08	16:55 – 17:20	08:36 – 08:58	17:28 – 17:52	09:19 – 09:40	> 32
5	27th Sep. – 2th Oct. 08	14:53 – 15:16	08:53 – 09:15	15:37 – 16:00	09:41 – 10:00	> 32
6	2th – 7th Oct. 08	14:45 - 15:07	10:06 – 10:36	15:33 – 15:55	11:02 – 11:20	< 22
7	7th – 12th Oct. 08	19:16 – 19:40	09:06 – 09:27	20:03 – 20:25	11:01 – 11:21	< 22
8	12th – 17th Oct. 08	16:12 – 16:48	09:31 – 09:52	17:04 – 17.27	10:43 – 11:05	< 22
9	17th – 22th Oct. 08	16:13 – 16:38	09:50 – 10:13	16:51 – 17:13	10:56 – 11:17	< 22
10	22th – 27th Oct. 08	15:39 – 16:04	09:59 – 10:21	16:19 – 16:51	10:54 – 11:14	< 22
11	27th Oct. – 1th Nov. 08	16:09 – 16:34	09:47 – 10:08	16:54 – 17:20	10:39 – 11:02	> 32
12	1th – 6th Nov. 08	15:03 – 15:27	10:08 – 10:30	15:44 – 16:06	11:06 - 11:29	> 32
13	6th – 11th Nov. 08	15:08 – 15:36	10:04 – 10:27	15:43 – 16:06	11:05 – 11:26	> 32

In addition, flow rate were recorded every fifth minutes during the tests cycles. Flow meter was placed outside the RWO container and was read by the RWO personell or NIVA personell, and immediately communicated to NIVAs site responsible. The averages of multiple flow measurements are given in **Table 7**. Flowrates were also calculated from the time for start/stop and the volume of the tanks, which give an accurate flow estimate. The ranges of calculated flowrates for all test cycles are given in the lower row of **Table 7**. As observed, the measured flow rates are in general in the lower end of the calculated ranges, indicating that the flowmeter slightly underestimated the actual flows.

Table 7. Average flowrates based on measurements and calculations (lower row)

Test cycle	Dates	Average flow ballasting m^3/h	Average flow deballasting m^3/h	Average flow control ballasting m^3/h	Average flow control deballasting m^3/h
1	3th – 8th Sep. 08	510	513	512	514
2	10th – 15th Sep. 08	516	511	509	511
3	17th – 22th Sep. 08	509	511	515	513
4	22th – 27th Sep. 08	510	507	512	512
5	27th Sep. – 2th Oct. 08	513	512	514	517
6	2th – 7th Oct.08	511	510	513	516
7	7th – 12th Oct. 08	509	510	514	522
8	12th – 17th Oct. 08	512	509	522	524
9	17th – 22th Oct. 08	507	509	513	519
10	22th – 27th Oct. 08	516	510	517	525
11	27th Oct. – 1th Nov. 08	508	509	518	524
12	1th – 6th Nov. 08	509	508	522	520
13	6th – 11th Nov. 08	510	506	519	526
Flow range for all test cycles based on calculations		471 - 600	400 - 600	485 - 573	522 - 667

3.3 Chemical water quality criteria

The temperature, salinity, pH, dissolved oxygen content and concentration of free and total chlorine (or oxidants) were measured in the test water tank (WST) prior to treatment, upon completion of the filling of the tanks and at day 1, 2 and 5 (before and after deballasting) in the storage tanks TT1 and CT2 in each test cycle. The results are given in **Table 8**. Measurements of redox were done but not reported in test cycle 1, due to problems with the electrode. To increase the confidence of the redox measurements, it was decide to do all redox measurements with two extra electrodes (as reported in **appendix G** – Logging of *in situ* measurements). The most reasonable results were obtained with the Walchem Wbh320-5nn controller with a pH/ORP Thermo Orion electrode, as described in **Chapter 2.6.1** "In situ measurements"**.** Redox measurements done with this electrode are therefore only shown in this report.

The results for salinity show that the requirement for the two testwater qualities to be separated by at least 10 PSU is fulfilled.

All results for TRO are given as average values of three replicate measurements (n = 3) ± standard deviation. The operational range of the DPD method for TRO measurements is from 0.02 to 2.0 mg/l Cl_2. Values below detection limit is shown in the table as < 0.02 mg/l Cl_2. The accuracy of measurements is reported to be ±0.02 mg/l in pure water, but the accuracy may be poorer due to interference in water with high particle content. This may explain unexpected high readings in control water in some of the test cycles. The background values of TRO in the test water (WST) and in the untreated water (control) was generally very low (0.02 – 0.08 mg/l Cl_2) and in accordance with what is expected to find in natural seawater outside Solbergstrand.

Table 8. Temperature, salinity, pH, redox, dissolved oxygen and free and total chlorine measurements in the test water tank (WST) prior to treatment and in the storage tanks TT1 (treated water) and CT2 (control water) for each test cycle. (b.d = before deballasting, a.d = after deballasting).

		WST	TT1							CT2						
	Day	**0**	**0**	**1**	**2**	**3**	**4**	**5 (b.d)**	**5 (a.d)**	**0**	**1**	**2**	**3**	**4**	**5 (b.d.)**	**5 (a.d)**
Parameter	**Unit**															
Test cycle 1																
Temperature	ºC	11.3	11.6	11.8	11.8	-	-	12.2	12.4	11.7	11.7	11.8	-	-	12.2	12.2
pH	-	7.86	7.96	7.90	7.89	-	-	7.87	7.92	7.93	7.93	7.90	-	-	7.84	7.84
Dissolved O_2	mg/l	10.1	9.7	9.1	9.6	-	-	9.4	10.8	9.8	9.1	9.1	-	-	8.5	8.5
Salinity	PSU	32.3	32.1	32.1	32.1	-	-	33.5	33.5	32.3	32.3	32.3	-	-	33.8	33.8
Redox	mV															
TRO as Free chlorine	mg/l Cl_2	0.07± 0.01	0.83± 0.02	0.16±0.02	0.09± 0.02	-	-	0.07± 0.02	1.97± 0.01	0.08± 0.02	0.05±0.01	0.05± 0.01	-	-	<0.02	0.05±0.05
TRO as Total chlorine	mg/l Cl_2	0.08±0.02	0.96± 0.02	0.24± 0.01	0.12± 0.01	-	-	0.04 ±0.03	2.05±0.02	0.14±0.02	0.07±0.01	0.07±0.01	-	-	0.04±0.02	0.07±0.06
Test cycle 2																
Temperature	ºC	10.5	10.7	10.8	10.9	-	-	10.8	11.0	10.7	10.7	10.9	-	-	10.8	10.8
pH	-	7.92	7.90	7.90	7.91	-	-	7.90	7.87	7.94	7.95	7.94	-	-	7.90	7.93
Dissolved O_2	mg/l	11.1	10.4	10.7	10.9	-	-	10.9	11.4	10.4	9.6	9.5	-	-	8.6	10.0
Salinity	PSU	32.5	32.3	32.3	32.3	-	-	32.3	32.3	32.5	32.5	32.5	-	-	32.6	32.5
Redox	mV	227	180.	146	135	-	-	177	167	240	238	235	-	-	228.5	237
TRO as Free chlorine	mg/l Cl_2	0.04±0.01	1.46±0.02	0.21±0.01	0.37±0.46	-	-	0.07±0.01	1.26±0.04	0.05±0.01	0.02±0.01	0.06±0.01	-	-	0.03±0.01	0.06±0.01
TRO as Total chlorine	mg/l Cl_2	0.06±0.01	1.71±0.06	0.31±0.01	0.15±0.01	-	-	0.12±0.01	1.47±0.03	0.10±0.01	0.04±0.01	0.07±0.01	-	-	0.04 ±0.01	0.09±0.01

		WST	TT1							CT2						
	Day	**0**	**0**	**1**	**2**	**3**	**4**	**5 (b.d)**	**5 (a.d)**	**0**	**1**	**2**	**3**	**4**	**5 (b.d.)**	**5 (a.d)**
Parameter	**Unit**															
Test cycle 3																
Temperature	ºC	9.8	10.0	10.0	10.2	-	-	10.1	10.3	9.9	10.1	10.1	-	-	10.1	10.4
pH	-	7.90	7.83	7.89	7.92	-	-	7.87	7.83	7.90	7.97	7.97	-	-	7.87	7.83
Dissolved O_2	mg/l	10.1	10.4	10.5	10.3	-	-	10.9	11.0	9.9	10.3	9.9	-	-	9.1	9.1
Salinity	PSU	32.6	32.4	32.6	32.6	-	-	32.6	32.6	32.6	32.5	32.6	-	-	32.6	32.6
Redox	mV	232	141.	90	132	-	-	171	164	250	227	265	-	-	235	242
TRO as Free chlorine	mg/l Cl_2	0.06±0.01	1.02±0.01	0.14± 0.01	0.07±0.02	-	-	0.05±0.01	0.87±0.04	0.06±0.01	0.04±0.01	0.07±0.01-	-		0.05±0.01	0.07±0.01
TRO as Total chlorine	mg/l Cl_2	0.07±0.01	1.18±0.04	0.22±0.02	0.16±0.01	-	-	0.09±0.01	0.99±0.04	0.08±0.01	0.05±0.01	0.09±0.01	-	-	0.06±0.01	0.07±0.01
Test cycle 4																
Temperature	ºC	9.0	9.2	9.3	9.2	9.2	9.4	9.4	9.4	9.2	9.3	9.2	9.2	9.6	9.4	9.5
pH	-	7.95	7.82	7.88	7.90	7.94	7.94	8.01	8.0	8.04	7.98	7.88	7.75	7.88	7.91	7.92
Dissolved O_2	mg/l	10.0	9.1	9.0	9.0	9.4	8.7	8.6	8.8	9.2	9.0	8.4	8.5	7.4	7.4	7.6
Salinity	PSU	33.1	32.9	32.8	32.9	32.9	32.9	32.9	32.9	33.1	32.8	33.0	33.1	33.1	33.1	32.9
Redox	mV	261	107	134	134	130	163	233	211	193	134	239	238	271	272	230
TRO as Free chlorine	mg/l Cl_2	0.02±0.01	0.93±0.02	0.10±0.01	0.06 ±0.01	-	-	0.03±0.01	1.13±0.16	< 0.02	0.02±0.01	0.04± 0.01	-	-	<0.02	0.13±0.04
TRO as Total chlorine	mg/l Cl_2	0.06±0.05	1.04±0.03	0.19±0.01	0.13±0.02	-	-	0.04±0.01	0.96±0.08	0.03±0.02	0.04±0.01	0.05±0.01	-	-	0.02±0.01	<0.02

		WST	TT1							CT2						
	Day	**0**	**0**	**1**	**2**	**3**	**4**	**5 (b.d)**	**5 (a.d)**	**0**	**1**	**2**	**3**	**4**	**5 (b.d.)**	**5 (a.d)**
Parameter	**Unit**															
Test cycle 5																
Temperature	ºC	8.7	8.9	10.4	8.9	8.8	-	8.7	8.6	8.9	10.3	8.8	8.7	-	8.7	8.7
pH	-	8.02	7.80	7.82	7.97	7.95	-	7.93	7.96	7.80	7.77	8.01	7.94	-	7.94	7.95
Dissolved O_2	mg/l	9.3	9.1	8.9	9.1	9.2	-	9.1	8.7	9.3	8.2	8.7	9.1	-	8.1	8.5
Salinity	PSU	33.5	33.4	33.1	33.4	33.4	-	33.4	33.4	33.5	33.3	33.5	33.5	-	33.4	33.0
Redox	mV	265	143		157	175	-	205	142	236		241	256	-	264	269
TRO as Free chlorine	mg/l Cl_2	0.09± 0.04	1.08±0.03	0.15±0.02	0.10±0.01	-	-	0.09±0.02	0.82±0.01	0.10±0.06	0.02±0.02	0.03±0.01	-	-	0.03±0.01	0.08±0.01
TRO as Total chlorine	mg/l Cl_2	0.05±0.01	1.12±0.05	0.27±0.01	0.16±0.03	-	-	0.06±0.01	0.92±0.03	0.08±0.01	0.02±0.01	0.04±0.01	-	-	0.05±0.02	0.18±0.02
Test cycle 6*																
Temperature	ºC	11.9	12.0	11.8	11.3	-	-	10.2	10.3	12.0	11.6	11.3	-	-	10.1	10.2
pH	-	8.17	8.03	8.04	7.93	-	-	7.85	7.80	8.01	7.99	7.96	-	-	7.99	7.99
Dissolved O_2	mg/l	8.1	8.1	8.7	8.2	-	-	6.5	6.80	8.1	8.1	7.8	-	-	6.9	7.2
Salinity	PSU	20.3	20.3	20.3	20.4	-	-	20.3	20.2	20.3	20.3	20.4	-	-	20.3	20.3
Redox	mV	275	166	117	140	-	-	166.5	142	235	240.	282	-	-	336.5	295
TRO as Free chlorine	mg/l Cl_2	0.02 ±0.02	0.28 ±0.01	0.03 ±0.03	<0.02	-	-	<0.02	0.26 ±0.01	0.02 ±0.02	0.06 ±0.02	<0.02	-	-	<0.02	<0.02
TRO as Total chlorine	mg/l Cl_2	0.03 ± 0.01	0.35 ±0.01	0.09 ± 0.02	<0.02	-	-	0.04 ± 0.02	0.39 ±0.02	0.03±0.03	0.08 ±0.01	< 0.02	-	-	<0.02	<0.02

		WST	TT1							CT2						
	Day	**0**	**0**	**1**	**2**	**3**	**4**	**5 (b.d)**	**5 (a.d)**	**0**	**1**	**2**	**3**	**4**	**5 (b.d.)**	**5 (a.d)**
Parameter	**Unit**															
Test cycle 7*																
Temperature	ºC	10.9	10.9	11.0	11.1	-	-	11.	10.9	10.8	10.8	11.0	-	-	10.7	10.9
pH	-	8.07	7.93	7.86	7.80	-	-	7.7	7.7	8.03	7.92	7.83	-	-	7.82	7.91
Dissolved O_2	mg/l	8.4	8.3	8.3	8.6	-	-	7.5	7.6	8.8	8.0	7.8	-	-	7	7.5
Salinity	PSU	21.4	21.6	21.4	21.4	-	-	21.4	21.4	21.6	21.4	21.4	-	-	21.4	21.4
Redox	mV	276	129	128	124	-	-	132.5	122	228	294	230	-	-	269.5	269.5
TRO as Free chlorine	mg/l Cl_2	0.08 ±0.01	0.25±0.01	0.07 ±0.02	0.08 ± 0.02	-	-	<0.02	0.28±0.02	<0.02	<0.02	<0.02	-	-	<0.02	0.10 ±0.0
TRO as Total chlorine	mg/l Cl_2	0.12±0.02	0.36±0.02	0.10 ±0.01	<0.02	-	-	<0.02	0.40 ±0.02	0.05± 0.01	0.04 ±0.01	0.03±0.01	-	-	<0.02	0.10±0.02
Test cycle 8*																
Temperature	ºC	11.5	11.8	11.6	11.4	-	-	10.2	10.1	11.5	11.6	11.2	-	-	10.0	10.1
pH	-	8.21	81.30	8.08	79.70	-	-	7.72	7.64	8.21	8.15	8.00	-	-	7.80	7.79
Dissolved O_2	mg/l	8.9	8.7	9.1	8.4	-	-	6.6	6.9	8.7	8.7	7.7	-	-	6.5	7.1
Salinity	PSU	21.0	21.0	21.0	21.0	-	-	21.0	20.9	21.0	21.0	21.0	-	-	21.0	21.0
Redox	mV	284	151	170	160	-	-	157	137	236	223	265	-	-	265	283
TRO as Free chlorine	mg/l Cl_2	0.19 ±0.01	0.31±0.01	0.10 ±0.02	0.02 ±0.01	-	-	0.02± 0.01	0.31±0.02	0.15 ±0.05	0.17 ±0.02	< 0.02	-	-	< 0.02	<0.02
TRO as Total chlorine	mg/l Cl_2	0.20±0.0	0.39± 0.01	0.12 ± 0.01	0.07±0.02	-	-	0.05±0.00	0.40±0.02	0.12 ±0.01	0.20 ±0.0	0.02±0.01	-	-	< 0.02	0.02 ±0.01

		WST	TT1							CT2						
	Day	**0**	**0**	**1**	**2**	**3**	**4**	**5 (b.d)**	**5 (a.d)**	**0**	**1**	**2**	**3**	**4**	**5 (b.d.)**	**5 (a.d)**
Parameter	**Unit**															
Test cycle 9																
Temperature	ºC	10.2	10.3	9.8	9.6	-	-	9.4	9.4	10.3	9.9	9.6	-	-	9.3	9.4
pH	-	8.11	8.06	8.21	8.04	-	-	7.8	7.77	8.12	8.07	8.05	-	-	7.89	7.85
Dissolved O_2	mg/l	8.5	8.4	7.5	7.8	-	-	7.4	7.6	8.4	8.6	8.5	-	-	8.0	8.0
Salinity	PSU	20.9	20.9	20.9	20.9	-	-	20.9	20.9	20.9	20.9	20.9	-	-	20.9	20.9
Redox	mV	279	141	150	139	-	-	130	71	276	269	225	-	-	262	274
TRO as Free chlorine	mg/l Cl_2	0.05±0.01	0.19 ± 0.01	0.02 ±0.01	0.02±0.01	-	-	0.02 ±0.0	0.27± 0.01	0.02 ± 0.01	0.02± 0.01	<0.02	-	-	< 0.02	<0.02
TRO as Total chlorine	mg/l Cl_2	0.06±0.02	0.30±0.01	0.02 ±0.01	0.03 ±0.01	-	-	0.03 ±0.01	0.42 ±0.02	0.03± 0.01	0.04±0.01	0.10 ±0.10	-	-	0.03 ±0.01	<0.02
Test cycle 10																
Temperature	ºC	10.1	10.3	9.8	9.9	-	-	9.4	9.5	10.1	9.7	9.8	-	-	9.4	9.4
pH	-	8.11	8.04	8.01	8.02	-	-	7.77	7.74	8.14	8.13	8.08	-	-	7.91	7.92
Dissolved O_2	mg/l	9.3	9.8	9.8	9.0	-	-	7.4	7.6	9.5	9.0	7.9	-	-	8.2	8.6
Salinity	PSU	21.1	21.1	21.1	21.1	-	-	21.1	21.1	21.1	21.1	21.1	-	-	21.1	21.1
Redox	mV	271	83	81	108	-	-	284	190	155	241	257	-	-	265	273
TRO as Free chlorine	mg/l Cl_2	<0.02	0.19±0.0	0.02 ± 0.01	<0.02	-	-	0.05 ±0.02	0.29 ±0.03	<0.02	< 0.02	< 0.02			< 0.02	< 0.02
TRO as Total chlorine	mg/l Cl_2	0.03±0.01	0.30 ± 0.02	0.07±0.02	0.05±0.04	-	-	0.08±0.06	0.40±0.02	0.02 ±0.01	0.02±0.01	< 0.02	-	-	< 0.02	< 0.02

		WST	TT1							CT2						
	Day	**0**	**0**	**1**	**2**	**3**	**4**	**5 (b.d)**	**5 (a.d)**	**0**	**1**	**2**	**3**	**4**	**5 (b.d.)**	**5 (a.d)**
Parameter	**Unit**															
Test cycle 11																
Temperature	ºC	10.6	10.9	10.2	9.5	-	-	8.8	7.8	10.7	10.1	9.2	-	-	7.6	7.5
pH	-	8.12	8.06	8.04	8.08	-	-	8.03	8.04	8.14	8.10	8.10	-	-	8.06	8.08
Dissolved O_2	mg/l	9.1	9.2	8.9	8.0	-	-	10.7	10.5	8.9	8.9	8.6	-	-	9.8	9.7
Salinity	PSU	32.8	32.3	32.3	32.3	-	-	32.3	32.2	32.4	32.4	32.3	-	-	32.3	32.3
Redox	mV	272	146.5	231.5	216.5	-	-	177	121	217	212	210.5	-	-	222	218.5
TRO as Free chlorine	mg/l	<0.02	0.75 ±0.02	0.06 ±0.01	0.05 ±0.01	-	-	0.03 ±0.02	0.02± 0.01	0.02 ± 0.01	<0.02	<0.02	-	-	<0.02	<0.02
TRO as Total chlorine	mg/l	<0.02	0.89 ± 0.01	0.11 ±0.01	0.08 ±0.01	-	-	0.05 ±0.01	<0.02	0.02±0.02	0.02 ±0.02	0.02 ± 0.02	-	-	<0.02	<0.02
Test cycle 12																
Temperature	ºC	9.5	9.6	8.5	8.2	-	-	7.1	7.0	9.4	8.5	8.2	-	-	6.7	6.8
pH	-	8.12	8.09	8.13	8.10	-	-	8.09	8.10	8.17	8.70	8.10	-	-	8.11	8.09
Dissolved O_2	mg/l	10.4	10.1	9.5	9.9	-	-	9.6	10.7	10.4	8.7	9.9	-	-	10.1	10.1
Salinity	PSU	32.2	32.2	32.2	32.2	-	-	32.2	32.2	32.2	32.2	32.2	-	-	32.2	32.2
Redox	mV	245	190	208.5	172	-	-	163	161	281	302	172	-	-	221.5	275
TRO as Free chlorine	mg/l	<0.02	0.87 ± 0.06	0.10 ±0.01	0.03± 0.01	-	-	0.02 ± 0.01	0.69 ±0.01	0.03 ± 0.01	<0.02	<0.02	-	-	<0.02	<0.02
TRO as Total chlorine	mg/l	<0.02	0.91±0.01	0.11 ±0.0	0.07 ± 0.02	-	-	0.04 ±0.01	0.84 ±0.01	<0.02	<0.02	0.02 ±0.01	-	-	0.02±0.01	0.03 ±0.01

		WST	TT1							CT2						
	Day	**0**	**0**	**1**	**2**	**3**	**4**	**5 (b.d)**	**5 (a.d)**	**0**	**1**	**2**	**3**	**4**	**5 (b.d.)**	**5 (a.d)**
Parameter	**Unit**															
Test cycle 13																
Temperature	ºC	9.2	9.3	9.1	8.7	-	-	8.1	8.2	9.3	9.1	8.6	-	-	8.0	8.0
pH	-	8.13	8.09	8.07	8.07	-	-	8.01	7.99	8.16	8.08	8.11	-	-	8	8.00
Dissolved O_2	mg/l	8.7	8.4	8.0	7.7	-	-	8.6	8.6	8.4	8.7	8.4	-	-	7.8	8.1
Salinity	PSU	32.4	32.5	32.4	32.4	-	-	32.4	32.4	32.4	32.4	32.4	-	-	32.4	32.4
Redox	mV	205	183.5	172.5	187.5	-	-	162	149.5	228.5	194	231	-	-	183.5	196
Free chlorine	mg/l	<0.02	1.06 ±0.01	0.11 ± 0.01	0.04 ±0.01	-	-	<0.02	0.72 ±0.02	0.03 ±0.01	<0.02	<0.02	-	-	<0.02	<0.02
Total chlorine	mg/l	0.03 ±0.01	1.19± 0.00	0.20 ± 0.01	0.08 ±0.00	-	-	0.03± 0.01	0.76 ± 0.06	0.06 ± 0.01	< 0.02	0.02 ± 0.01	-	-	<0.02	<0.02

***** TRO values corrected for erroneous sample blank. TRO measurements for three test cycles were performed with distilled water as blank. As this was not in accordane with the test procedure, all values were therefore corrected by subtracting the results obtained using distilled water with a correction factor i.e. subtracting the background level as measured in a number of sample blanks.

3.3.1 Decay study of TRO

After deballasting on day 5, a decay study of the TRO level in treated water was conducted. TRO was measured as the concentration of free and total clorine. The results for decay study of TRO in seawater are given in **Figure 2a** and **2b** and the results for decay study of TRO in brackish water are given in **Figure 3a** and **3b**. Test cycle 11 was run with a neutralization step (sodium thiosulphate) included in the deballasting process. All TRO decay values recorded for this test was below the sensitivity of the DPD method (0.02 mg/l Cl_2).

The TRO values measured immediately after deballasting in test cycle 1 and 2 (respectively 2.05 and 1.47 mg/l Cl_2 TRO measured as totale chlorine) were higher than the other seawater tests (TRO measured as total chlorine in the range 0.76 – 0.96 mg/l Cl_2) and the decay curves for these two tests (1 and 2) deviates from the other test results. As mentioned above, at least test cycle 1 is not representative for the normal operation of the CleanBallast system. The shape of the decay curve, is however, relatively consistent for all test cycles, i.e. a relatively rapid TRO decay during the first 4 hours and then a gradual decline towards the end of the observation period (48 h). In the brackish water tests **(Figure 3a** and **3b**), the TRO value recorded immediately after deballasting was stable for all tests (range: 0.39 – 0.42 mg/l Cl_2 as totale chlorine), but lower than in the seawater tests. This was expected due to the lower chloride concentration and therefore lower initial production of TRO by the electrolytic unit and the higher concentration of chlorine demanding substances (i.e. organic matter) in brackish water. The shape of the decay curves were consistent for all brackish water tests, with a relatively rapid decay during the first 4 hours, and than a flattening of the curve for the rest of the observation period.

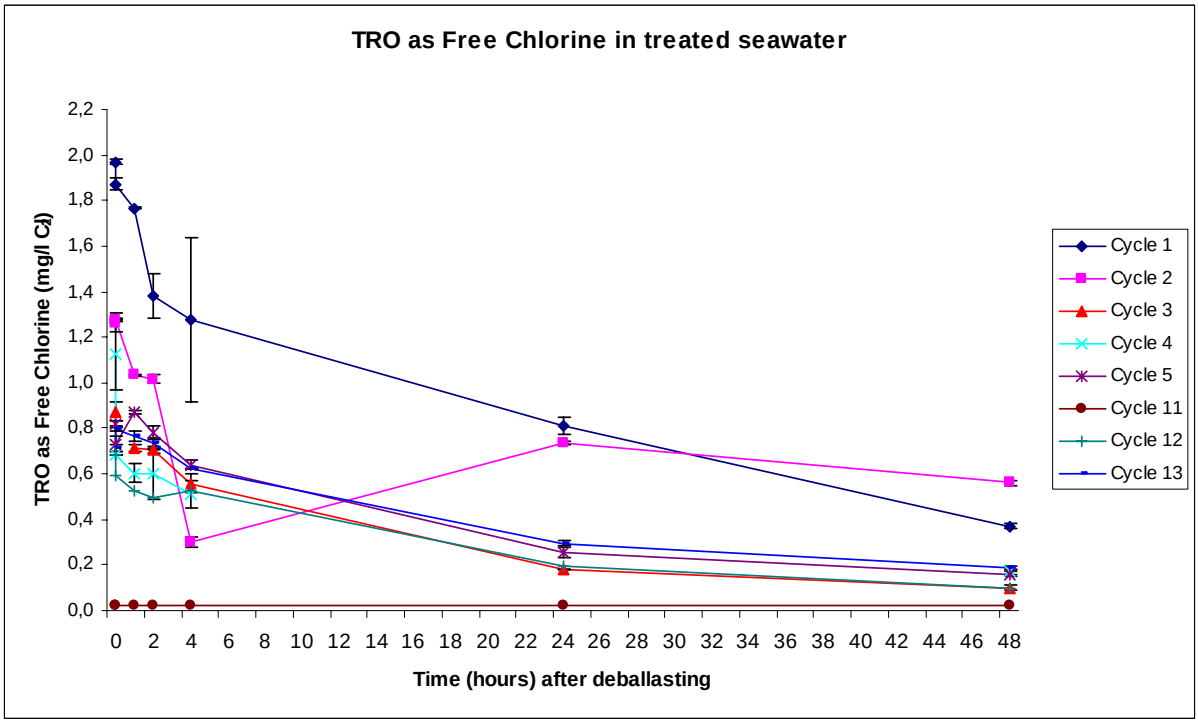

Figure 2a. Test cycle 1-5 and 11-13. Concentration of TRO measured as free chlorine (mg/l Cl_2) in treated seawater during a 48 h period, starting on day 5 immediately after deballasting. Measurements of TRO 30 min after deballasting in test 3 is missing. Measurements of TRO 24 h after deballasting in test 4 is missing. All results are shown as average of three measurements ± std.

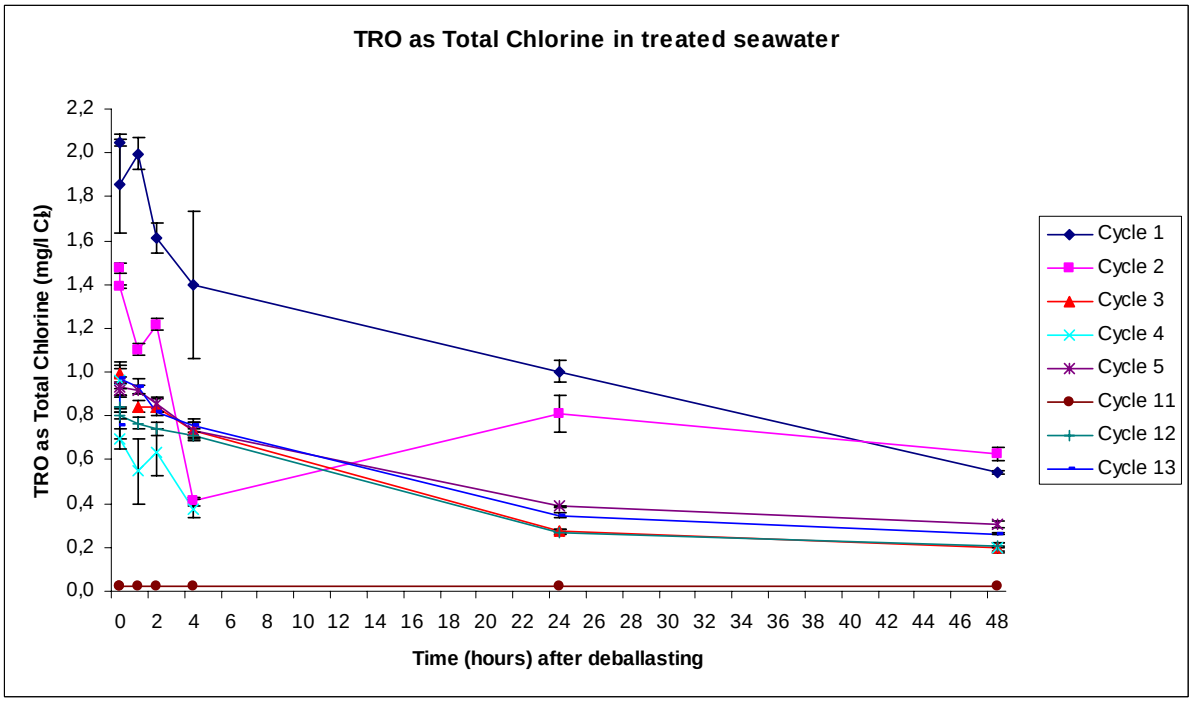

Figure 2b. Test cycle 1-5 and 11-13. Concentration of TRO measured as total chlorine (mg/l Cl_2) in treated seawater during a 48 h period, starting on day 5 immediately after deballasting. Measurements of TRO 30 min after deballasting in test 3 is missing. Measurements of TRO 24 h after deballasting in test 4 is missing. All results are shown as average of three measurements ± std.

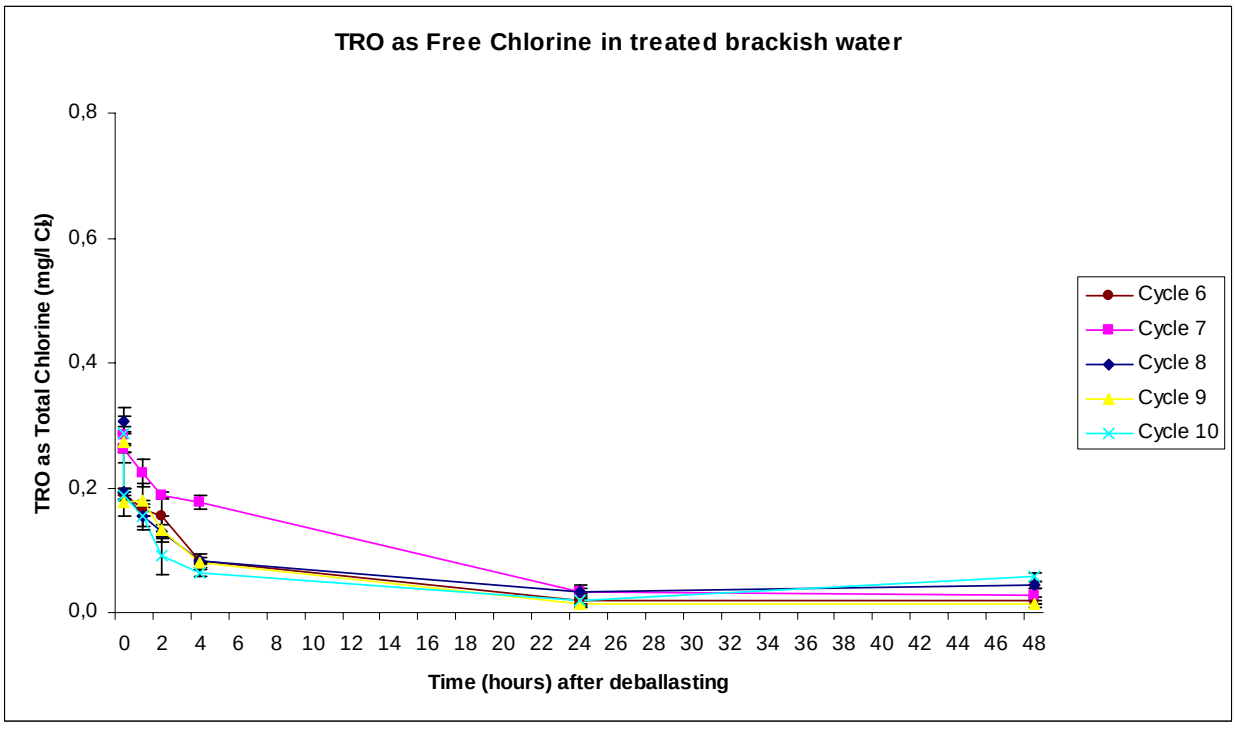

Figure 3a. Test cycle 6-10. Concentration of TRO measured as free chlorine (mg/l Cl_2) in treated brackish water during a 48 h period, starting on day 5 immediately after deballasting. All results are shown as average of three measurements ± std.

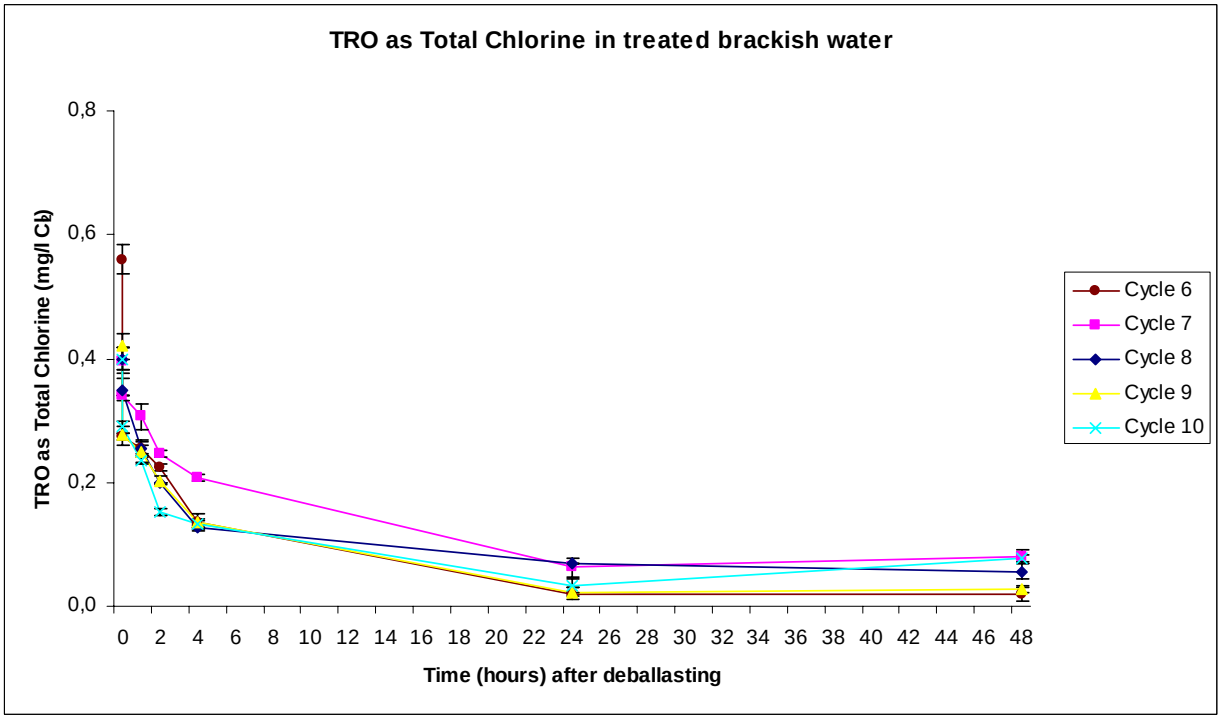

Figure 3b. Test cycle 6-10. Concentration of TRO measured as total chlorine (mg/l Cl_2) in treated brackish water during a 48 h period, starting on day 5 immediately after deballasting. All results are shown as average of three measurements ± std.

3.3.2 Chemical water quality of test water

Concentration of total suspended solids (TSS), dissolved organic carbon (DOC) and particulate organic carbon (POC) at day 0 in WST, TT1 and CT2, and at day 5 in TT2 and TT1 in the different test cycles are shown in **Table 9**. The chemical water quality requirements were fulfilled for all tests.

Table 9. Chemical water quality (average and standard deviation of triplicate samples). Green background indicates that required level was fulfilled, yellow background partial fulfilment, while red background indicates failure to fulfil required level.

TEST 1	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 2.0 kg**		**$DOC_{Additive}$ = 2.5 kg**		**$POC_{Additive}$ = 3.0 kg**	
Required level, influent water	>1	-	>1	-	>1	-
Influent water (WST)	15.3	1.2	2.2	0.1	2.3	0.4
Treated day 0 (TT1)	15.0	1.0	2.2	0.1	1.1	0.5
Treated day 5 (TT2)	7.3	0.7	2.4	0.1	0.7	0.1
Control day 0 (CT2)	15.2	1.4	2.2	0.1	1.8	0.3
Control day 5 (CT2)	5.8	0.5	1.9	0.1	0.7	0.1
TEST 2	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 2.0 kg**		**$DOC_{Additive}$ = 2.5 kg**		**$POC_{Additive}$ = 3.0 kg**	
Required level, influent water	>1	-	>1	-	>1	-
Influent water (WST)	13.3	3.2	2.2	0.1	2.3	0.1
Treated day 0 (TT1)	11.7	0.9	2.2	0.1	2.0	0.0
Treated day 5 (TT2)	5.5	0.4	3.3	0.0	0.7	0.1
Control day 0 (CT2)	12.1	0.2	2.1	0.1	2.3	0.1
Control day 5 (CT2)	5.6	0.6	2.2	0.0	0.7	0.2
TEST 3	**TSS mg/l**		**DOC mg/l**		**POC,mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 2.0 kg**		**$DOC_{Additive}$ = 2.5 kg**		**$POC_{Additive}$ = 3.0 kg**	
Required level, influent water	>1	-	>1	-	>1	-
Influent water (WST)	16.9	3.3	2.4	0.1	2.5	0.3
Treated day 0 (TT1)	13.5	2.1	2.4	0.1	2.1	0.4
Treated day 5 (TT2)	9.1	0.3	1.9	0.1	1.5	0.2
Control day 0 (CT2)	12.1	0.8	2.3	0.1	2.4	0.0
Control day 5 (CT2)	5.4	0.3	2.8	0.1	0.0	0.0
TEST 4	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 2.0 kg**		**$DOC_{Additive}$ = 2.5 kg**		**$POC_{Additive}$ = 3.0 kg**	
Required level, influent water	>1	-	>1	-	>1	-
Influent water (WST)	11.6	1.0	2.2	0.1	2.2	0.3
Treated day 0 (TT1)	10.7	1.0	2.3	0.0	2.0	0.4
Treated day 5 (TT2)	8.9	1.7	2.7	0.1	0.7	0.02
Control day 0 (CT2)	11.1	0.3	2.3	0.1	2.3	0.1
Control day 5 (CT2)	7.2	2.5	2.0	0.0	0.7	0.02

TEST 5	TSS mg/l		DOC mg/l		POC mg/l	
	Average	Stdev	Average	Stdev	Average	Stdev
Additions to influent water	**$TSS_{Additive}$ = 2.0 kg**		**$DOC_{Additive}$ = 2.5 kg**		**$POC_{Additive}$ = 3.0 kg**	
Required level, influent water	>1	-	>1	-	>1	-
Influent water (WST)	13.3	0.9	2.0	0.1	2.1	0.2
Treated day 0 (TT1)	12.8	3.6	2.0	0.1	1.5	0.3
Treated day 5 (TT2)	10.6	4.0	2.5	0.1	0.7	0.0
Control day 0 (CT2)	14.2	1.7	2.0	0.1	1.5	0.3
Control day 5 (CT2)	6.7	1.8	1.9	0.1	0.7	0.0
TEST 6	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 20 kg**		**$DOC_{Additive}$ = 10 kg**		**$POC_{Additive}$ = 12.5 kg**	
Required level, influent water	>50	-	>5	-	>5	-
Influent water (WST)	57.4	0.6	5.8	0.1	6.7	0.7
Treated day 0 (TT1)	56.9	0.5	6.1	0.1	8.0	1.5
Treated day 5 (TT2)	20.2	2.6	5.9	0.0	1.2	0.2
Control day 0 (CT2)	55.5	1.0	6.0	0.1	6.3	0.6
Control day 5 (CT2)	9.3	1.6	5.6	0.2	0.9	0.2
TEST 7	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 20 kg**		**$DOC_{Additive}$ = 10 kg**		**$POC_{Additive}$ = 12.5 kg**	
Required level, influent water	>50	-	>5	-	>5	-
Influent water (WST)	64.1	0.6	6.6	0.1	6.7	0.4
Treated day 0 (TT1)	57.1	3.6	6.8	0.1	6.8	1.1
Treated day 5 (TT2)	24.3	0.4	6.0	0.1	1.4	0.0
Control day 0 (CT2)	60.5	2.0	6.7	0.1	6.7	0.9
Control day 5 (CT2)	18.3	5.8	5.7	0.1	1.2	0.2
TEST 8	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 20 kg**		**$DOC_{Additive}$ = 10 kg**		**$POC_{Additive}$ = 12.5 kg**	
Required level, influent water	>50	-	>5	-	>5	-
Influent water (WST)	64.4	2.9	6.9	0.2	7.4	0.2
Treated day 0 (TT1)	60.0	1.4	6.9	0.1	7.0	0.2
Treated day 5 (TT2)	25.0	2.0	5.6	0.5	1.4	0.1
Control day 0 (CT2)	59.7	1.0	7.2	0.3	7.2	0.3
Control day 5 (CT2)	12.7	0.5	5.7	0.1	1.1	0.1
TEST 9	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 20 kg**		**$DOC_{Additive}$ = 10 kg**		**$POC_{Additive}$ = 12.5 kg**	
Required level, influent water	>50	-	>5	-	>5	-
Influent water (WST)	63.4	0.3	6.5	0.7	7.8	0.8
Treated day 0 (TT1)	60.8	2.9	7.0	0.2	6.3	0.4
Treated day 5 (TT2)	15.2	0.7	5.6	1.0	1.0	0.1
Control day 0 (CT2)	60.9	1.3	6.6	0.5	6.8	0.2
Control day 5 (CT2)	10.0	0.3	5.9	0.0	1.3	0.0

TEST 10	TSS mg/l		DOC mg/l		POC mg/l	
	Average	Stdev	Average	Stdev	Average	Stdev
Additions to influent water	**$TSS_{Additive}$ = 20 kg**		**$DOC_{Additive}$ = 10 kg**		**$POC_{Additive}$ = 12.5 kg**	
Required level, influent water	>50	-	>5	-	>5	-
Influent water (WST)	54.3	1.2	6.2	0.2	9.0	0.3
Treated day 0 (TT1)	51.1	0.3	6.4	0.4	7.5	0.5
Treated day 5 (TT2)	13.2	0.6	6.3	0.1	0.9	0.0
Control day 0 (CT2)	53.0	0.9	6.1	0.1	8.5	0.2
Control day 5 (CT2)	13.3	0.2	6.4	0.3	1.4	0.1
TEST 11	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 2.0 kg**		**$DOC_{Additive}$ = 2.5 kg**		**$POC_{Additive}$ = 3.0 kg**	
Required level, influent water	>1	-	>1	-	>1	-
Influent water (WST)	14.6	4.0	2.1	0.1	2.6	0.1
Treated day 0 (TT1)	11.4	0.3	2.3	0.1	2.2	0.1
Treated day 5 (TT2)	8.9	2.9	2.6	0.1	0.7	0.0
Control day 0 (CT2)	12.4	1.9	2.2	0.1	2.8	0.2
Control day 5 (CT2)	8.8	0.9	2.3	0.1	0.8	0.0
TEST 12	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 2.0 kg**		**$DOC_{Additive}$ = 2.5 kg**		**$POC_{Additive}$ = 3.0 kg**	
Required level, influent water	>1	-	>1	-	>1	-
Influent water (WST)	15.7	0.5	3.0	0.2	2.3	0.1
Treated day 0 (TT1)	14.5	1.3	3.3	0.2	2.0	0.1
Treated day 5 (TT2)	7.4	0.8	2.5	0.1	0.9	0.1
Control day 0 (CT2)	15.6	0.6	3.1	0.2	2.2	0.1
Control day 5 (CT2)	6.1	0.9	2.1	0.1	0.6	0.1
TEST 13	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 2.0 kg**		**$DOC_{Additive}$ = 2.5 kg**		**$POC_{Additive}$ = 3.0 kg**	
Required level, influent water	>1	-	>1	-	>1	-
Influent water (WST)	13.1	3.3	2.2	0.1	2.4	0.1
Treated day 0 (TT1)	11.9	0.9	2.3	0.1	1.9	0.2
Treated day 5 (TT2)	4.9	0.8	2.4	0.1	0.7	0.0
Control day 0 (CT2)	14.5	0.6	2.3	0.1	2.4	0.2
Control day 5 (CT2)	3.9	1.1	2.1	0.0	0.5	0.0

3.3.3 Disinfection by-products

Relevant disinfection by-products were analysed for all test cycles. A summary of all results are shown in **Appendix 1** enclosed to this report. Disinfection by-products with concentrations above the detection limit are highlighted. The chemistry of chlorine and other oxidants in seawater is complex due to the variety of compounds that can be involved in redox-reactions. When chlorine compounds are applied to seawater, a variety of chemical compounds will be oxidised according to their redox potentials shown below (compounds with lower redox-potential will be oxidised). The potential for formation is largest for iodine and bromine. The low concentration of iodine in seawater (<1 mg/L) gives a low formation potential, although iodide may be completely oxidized. Bromide concentration around 60-70 mg L^{-1} in seawater gives a high formation potential of bromo-oxides. This implies that in chlorinated seawater, Br^- is oxidised to active bromine (HOBr and OBr^-). The reaction is fast, and within 10 seconds bromo-oxide compounds will becomes the primary

biocide (Harboe and Poleo, 1997). Chloramines and bromamines may also be formed in proportion to the amino-nitrogen concentration in the seawater.

$O_3 + 2H^+ + 2e^- \leftrightarrow O_2 + H_2O$ $E^o = +2.07$ V
$HOCl + H^+ + e^- \leftrightarrow ½\ Cl_{2(g)} + H_2O$ $E^o = +1.63$ V
$HOBr + H^+ + e^- \leftrightarrow ½\ Br_{2(l)} + H_2O$ $E^o = +1.59$ V
$½\ Cl_{2(g)} + e^- \leftrightarrow +Cl^-$ $E^o = +1.36$ V
$OBr^- + H_20 + 2e^- \leftrightarrow Br^- + 3HO^-$ $E^o = +0.76$ V

The total residual oxidants (TRO) formed in chlorinated seawater (mainly HOBr and $OBr^-$and small amounts of chloramines and bromamines) are more persistent than their parent compounds. However, the TRO will gradually degrade in redox-reactions with inorganic and organic matter, with the liberation of Br^--ions and formation of persistent halogenated organic by-products in low concentrations.

One group of halogenated organic compounds of concern is the trihalomethanes (THMs). The dominating THM in chlorinated seawater is bromoform, which is a probable human carcinogen (USEPA, 1996), known to have the potential to bio-accumulate in aquatic animals. Bromoform is moderately persistent in the aquatic environment with a half life of 20 to 200 days (Vincoli, 1997).

In the present study, treated water in all test cycles were sampled and analysed for AOX, EOX, bromate, trihalomethanes (THMs) and other halogenated organic compounds. These compounds were analysed to clarify if the residual oxidants produced did react with organic substances to potential harmful organic compounds, and in what concentrations. Results are given in **Appendix 1**.

As expected, the dominating halogenated organic compound, and THM, was bromoform. Immediately after deballasting on day 5, bromoform was detected in concentrations in the range from 96-240 µg/l for brackish water and 86-200 µg/l for seawater. For comparison, The WHO Guideline Drinking Water Value is 100 µg/L for bromoform (WHO, 2006). In general, halogenated organic compounds are not acute toxic to aquatic animals in the concentration range detected. Even the most toxic by-product, which is bromoform, has to be present in the mg-range to exibit an acute toxic effect. Bromoform has an NOEC for algae (the most sensitive trophic group) of ≥2 mg/l (Erickson and Hawkins, 1980).

Another compound of concern is bromate, which was found in concentrations up to 29 µg/l in brackish water and up to 19 µg/l in seawater. The bromate ion can not be further oxidized, and will be the final product of the oxidation of bromide ion in seawater. Bromate ion is a stable compound, and not acute toxic to aquatic animals. For fish early lifstages, a 96 h LC_{50} of 31 mg BrO_3/l of newly hatched larvae has been reported (Hutchinson et al., 1997). However, bromate is a suspected animal carcinogen (Kurokawa et al., 1986), listed as a probable human carcinogen by USEPA (1996). It was expected that some bromate would be formed in the process.

3.3.4 Chemical fate analysis of disinfection by-products

During the storage period of five days and after deballasting on day 5 in test cycle 2 (seawater) and test cycle 9 (brackish water), a study of the concentration of disinfection by-products in treated water was done. Measurements were done on day 0, 2 and 5 after first treatment (ballasting) and during a 48 h period, starting immediately after second treatment (deballasting). **Figure 3a** and **3b** and **Figure 4a** and **4b** shows the result from compounds that were detected in the treated water in test cycle 2 (seawater) and test cycle 9 (brackish water), respectively. An overview of all the results obtained from treated water and control water are shown in **Appendix 2** – Chemical fate study of disinfection by-products, test cycle 2 and test cycle 9.

The concentrations of the sum parameters AOX and EOX (**Figure 3a**) were surprisingly low compared to the concentrations of bromoform (**Figure 3b**) in the same test cycle. The adsorbable organically bound halogens and extractable organically bound halogens varied during the time span, but the values were in general lower than the bromoform-concentrations. The analytical procedure for AOX and EOX may suffer from interference of some non-halogenated compounds in complex solutions such as seawater and brackish water. The shape of the curves for AOX and EOX (**Figure 3a**) were almost identical, except for AOX detected on day 5 and 48 hours after day 5. AOX and EOX compounds are only analysed once and not reported with standard devation. It is therefore difficult to assess if this deviation is real or just within the variation of the analytical precision of the method.

In seawater, the majority of bromoform was formed during the 2 first days (**Figure 3b**). After that time, no increase or reduction was evident. In brackish water, the pattern was different with the highest increase after the second treatment during deballasting (**Figure 4b**). The second treatment also raised the concentrations of AOX and EOX (**Figure 4a**).

The concentrations of bromoform detected were not very high. With a dilution of 1:1 at discharge to the sea, the level will be in the range accepted for drinking water.

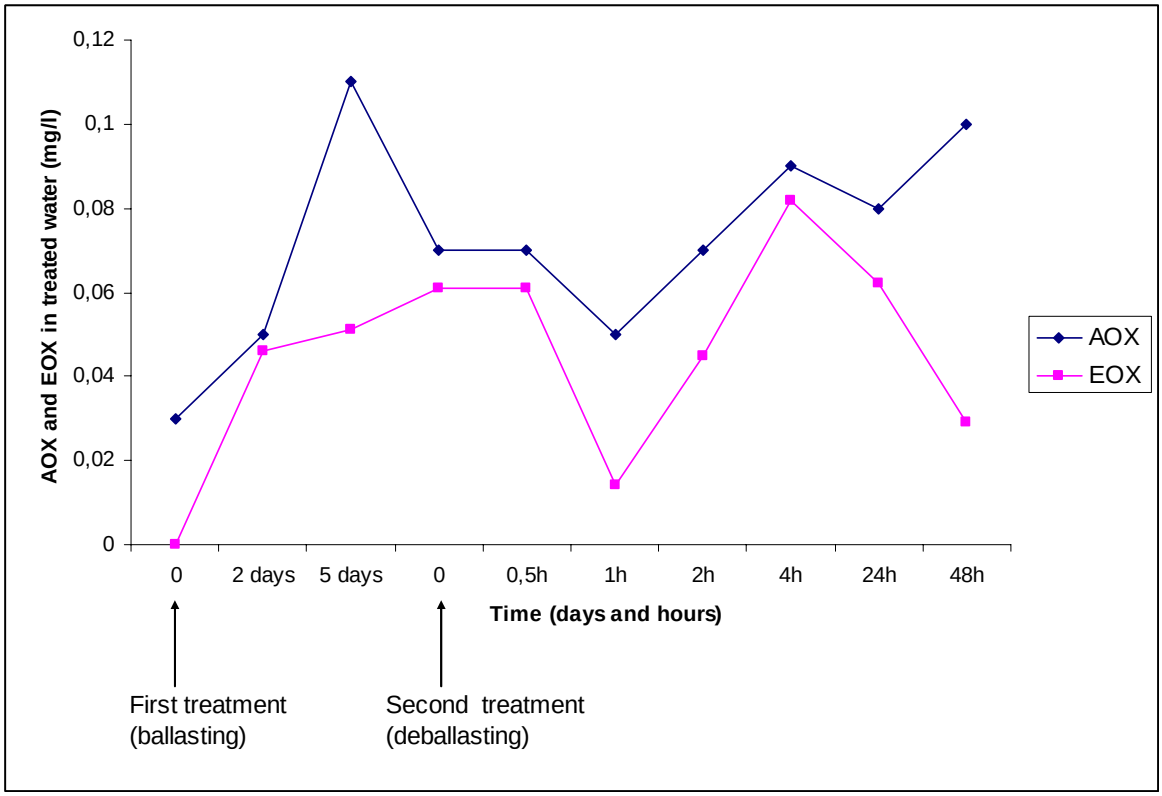

Figure 3a. Concentration of disinfection by-products (AOX and EOX) (mg/l) in treated seawater in test cycle 2, measured at defined days after ballasting and defined hours after deballasting.

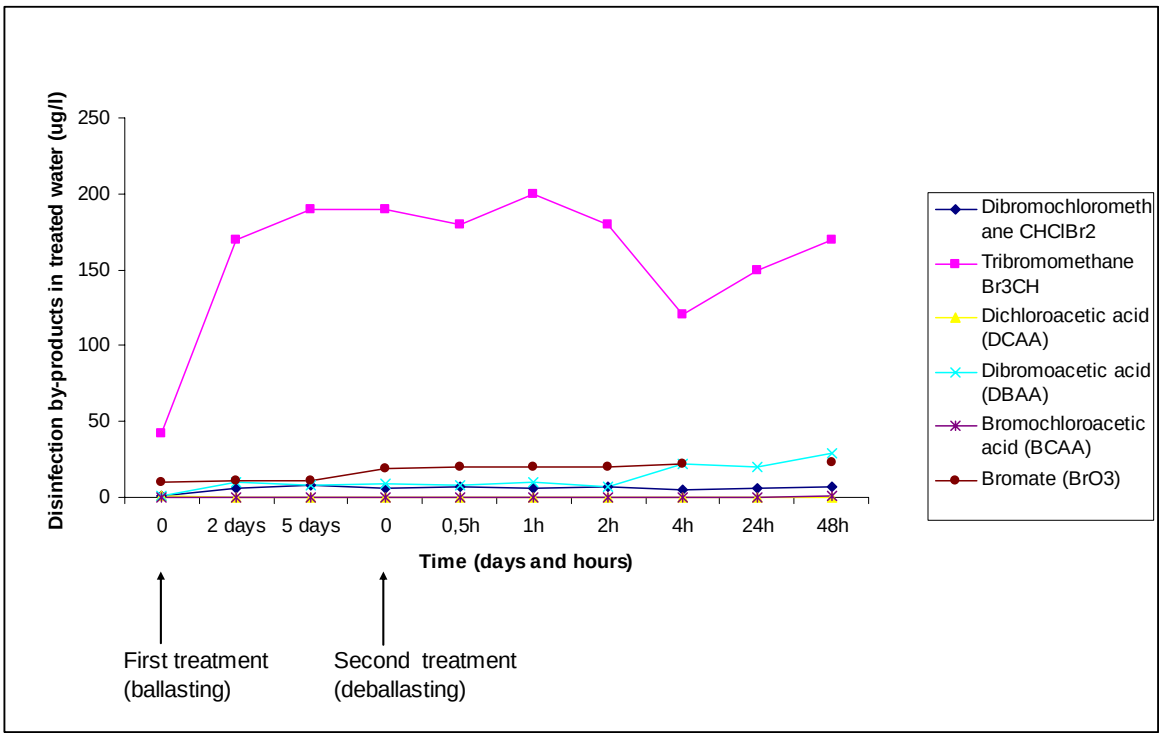

Figure 3b. Concentration of disinfection by-products (µg/l) in treated seawater in test cycle 2, measured at defined days after ballasting and defined hours after deballasting.

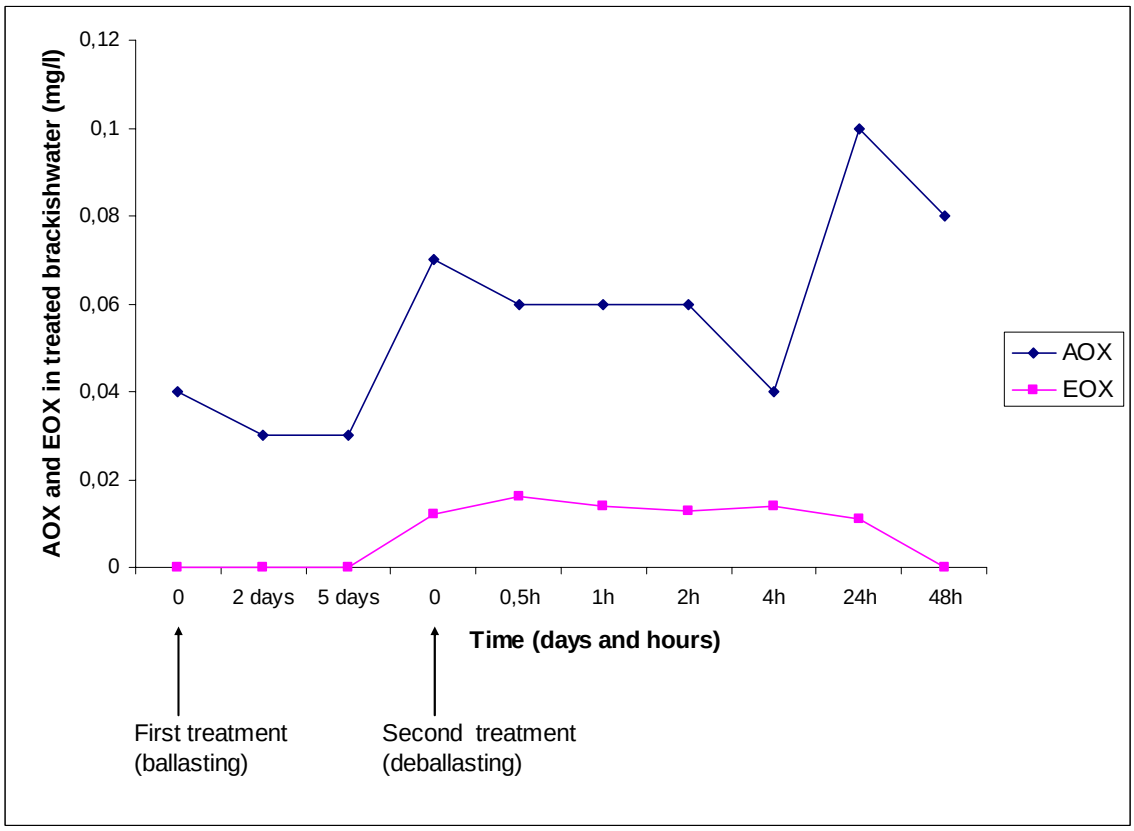

Figure 4a. Concentration of disinfection by-products (AOX and EOX) (mg/l) in treated brackish water in test cycle 9, measured at defined days after ballasting and defined hours after deballasting.

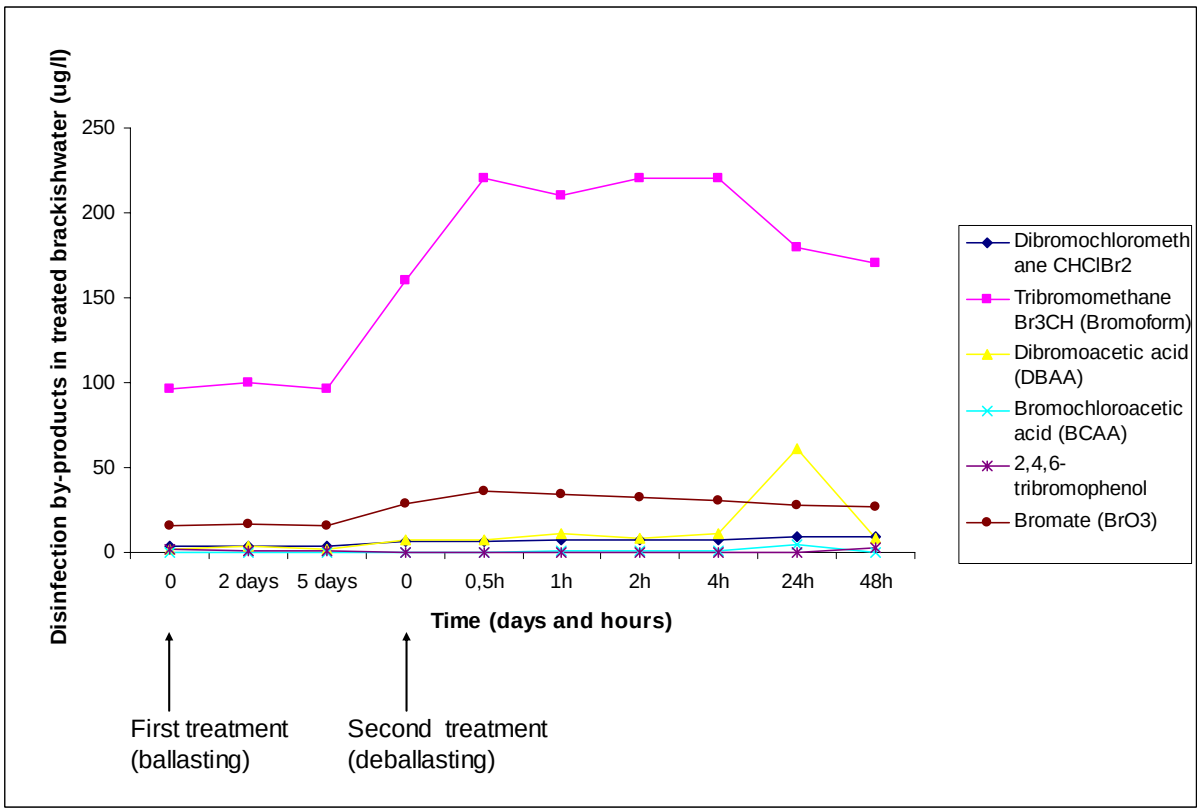

Figure 4b. Concentration of disinfection by-products (µg/l) in treated brackish water in test cycle 9, measured at defined days after ballasting and defined hours after deballasting.

3.3.5 Sludge characterization

On day 0 in test cycle 5 and 6, backwash water from the filter was sampled and analysed. Results are shown in **Table 10**.

Table 10. Characterization of sludge from filter backwash water

Parameter	Unit	Test 5, day 0	Test 6, day 0
Temperature	ºC	14.4	11.2
pH	PSU	7.83	7.89
Dissolved O_2	mg/l	6.9	8.9
Salinity	PSU	20.4	13.9
Redox	mV	nd	242
Total suspended solids (TSS)*	mg/l	20.8	59.0
Total organic carbon (TOC)*	mg/l	7.3	11.6
Dissolved organic carbon (DOC)*	mg/l	1.8	3.9
Particulate organic carbon (POC)*	mg/l	5.5	7.8
Total dry matter (TTS)*	g/l	21.2	15.3
Ignition loss*	%	69.9	45.9
Density*	g/ml	1.021	1.021
Turbidity*	FNU	17.8	51.4

* Average of duplicate determinations
nd – not determined

3.4 Gas measurements

Due to the possible formation of gas (hydrogen and oxygen) by the electrolytical EctoSys® disinfection in the CleanBallast system, a gas trap with a deaeration pipe was installed after the EctoSys® in order to remove free gas to the ambient air and prevent that gas can enter the ballast / storage tank with the effluent water of the CleanBallast system. Therefore, gas measurements were conducted upon ballasting and deballasting. During the tests, the gas volume leaving the deaeration pipe is trapped and lead to a measuring cyclinder. Previously the measuring cylinder was filled with drinking water and placed upside down in a container with drinking water. From the end of the dearation pipe the formed gas was lead via a pipe into the top of the upside down placed cylinder where the gas accumulates. The change in gas volume in the measuring cylinder was recorded (ml) during ballasting and deballasting operations. No differences indicated that no gas was produced during the test cycle. A positive difference indicated that gas was produced during the test cycle.

Table 11 shows the gas records for all test cycles. In test cycle 6 and 7 some gas development were recorded upon ballasting and deballasting. This coincided with the shift from seawater as test water to brackish water. No gas development was, however, recorded in any of the other test cycles. Gas measurements were not performed during test cycle 13.

Table 11. Gas measurements during test cycle 1-12 for treated water.

	Ballasting (treated water)			**Deballasting (treated water)**		
	Start gas recording	Stop gas recording	Difference	Start gas recording	Stop gas recording	Difference
Test cycle 1	150 ml	150 ml	0	150 ml	150 ml	0
Test cycle 2	110 ml	110 ml	0	110 ml	110 ml	0
Test cycle 3	120 ml	120 ml	0	120 ml	120 ml	0
Test cycle 4	120 ml	120 ml	0	100 ml	100 ml	0
Test cycle 5	110 ml	110 ml	0	40 ml	40 ml	0
Test cycle 6	80 ml	85 ml	5 ml	210 ml	210 ml	0
Test cycle 7	290 ml	295 ml	5 ml	140 ml	143 ml	3 ml
Test cycle 8	135 ml	135 ml	0	135 ml	135 ml	0
Test cycle 9	440 ml	440 ml	0	100 ml	100 ml	0
Test cycle 10	250 ml	250 ml	0	80 ml	80 ml	0
Test cycle 11	130 ml	130 ml	0	120 ml	120 ml	0
Test cycle 12	120 ml	120 ml	0	120 ml	120 ml	0

In addition to the oxygen and hydrogen gas concentration measurements in the deaeration pipe, which are automatically recorded by the CleanBallast system, manual measurements of hydrogen gas concentrations in two test cycles (test 9 and 12) were carried out with a portable analyser (Dräger PACIII; range 0-2000 ppm which equals 0-0.2 volume %) at different times (start, middle and end) for each operation (ballasting/deballasting) in the storage tank for treated water and control water. As shown in **Table 12**, the hydrogen gas concentrations in the closed treatment tank were very low and far beyond the explosion limit at ballasting and deballasting. This was expected due to the previous removal of free gas by the gas trap and deaeration pipe after the CleanBallast system.

Table 12. Hydrogen gas concentrations in the closed storage tank for treated water and control water during ballasting and deballasting operations.

		Treated water			**Control water**	
Test cycle	**Start**	**Middle**	**End**	**Start**	**Middle**	**End**
Cycle 9 (> 22 PSU)						
Ballasting	50 ppm	170 ppm	205 ppm	0 ppm	0 ppm	0 ppm
Deballasting	30 ppm	110 ppm	125 ppm	0 ppm	0 ppm	0 ppm
Cycle 12 (< 32 PSU)						
Ballasting	35 ppm	95 ppm	105 ppm	0 ppm	0 ppm	0 ppm
Deballasting	50 ppm	70 ppm	75 ppm	0 ppm	0 ppm	0 ppm

3.5 Fulfillment of the biological water quality criteria

The initial test waters' content of organisms should comply with the requirements in G8 as given in **Table 2.** Results from quantitative measurements of the initial test waters are given in **Table 13**.

Organisms ≥50 µm in minimum diameter
The requirements regarding density of the ≥50 µm group was met in all test cycles. The requirements regarding the biological diversity within the population were fulfilled.

Organisms ≥10-50 µm in minimum diameter
The quantifications of individuals in the ≥10-50 µm group were based on growth in dilution series, growth on agar plates and microscope counts of CFDA-AM stained cells. The requirements regarding influent density of the ≥10-50 µm group was met in all tests, for at least two of the three methods. For test 2 and 7 the requirement was not in compliance as measured by one of the growth methods, but in compliance when the more precise microscopic count was used.

Heterotrophic bacteria
The only requirement regarding bacteria in influent water is that heterotrophic bacteria should be present in a density of $\geq 10^4$ cfu ml^{-1}. This requirement was fulfilled in all tests as shown in **Table 13**.

The IMO G8 guideline 2.3.20 also specifies that "…the following bacteria do not need to be added to the influent water, but should be measured at the influent and at the time of discharge: 1. Coliform; 2. Enterococcus group; 3. *Vibrio cholerae* and 4. Heterotrophic bacteria." All these bacteria groups were measured in the influent water and the results are given in Table 13.

Table 13. Initial content of organisms within the defined test organism groups (ref. **Table 2**) in the test water in test 1-13. Green background indicates that required level was fulfilled, yellow background partial fulfilment, while red background indicates failure to fulfil required level.

Test organism	Method	Influent	Requirement
Test 1			
Organisms ≥50 µm	Microscope counts	193375	$\geq 10^5$ m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method	5000	≥1000 ml^{-1}
	95 % conf. interval	2000-20000	
	Microscope counts	2904	
	Plate counts	1650	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts	$2.1 \pm 0.08 \times 10^4$	$\geq 10^4$ cfu ml^{-1}
Coliform bacteria	Bacterial counts	0 ± 0	-
Vibrio sp.	Bacterial counts	$1.0 \pm 0.08 \times 10^4$	-
Vibrio cholerae	Bacterial counts, elimination method	-	-
Enterococcus group	Bacterial counts	$0.3 \pm 0.6 \times 10^0$	-

Test organism	Method	Influent	Requirement
Test 2			
Organisms ≥50 µm	Microscope counts	219308	≥10^5 m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method	900*	≥1000 ml^{-1}
	95 % conf. interval	300-3000	
	Microscope counts	1487	
	Plate counts	1250	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts	2.0 ± 0.0 x 10^4	≥10^4 cfu ml^{-1}
Coliform bacteria	Bacterial counts	0 ± 0	-
Vibrio sp.	Bacterial counts	6.5 ± 1.5 x 10^4	-
Vibrio cholerae	Bacterial counts, elimination method	-	-
Enterococcus group	Bacterial counts	1.0 ± 0.0 x 10^2	-
Test 3			
Organisms ≥50 µm	Microscope counts	217417	≥10^5 m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method	1100	≥1000 ml^{-1}
	95 % conf. interval	400-3000	
	Microscope counts	2368	
	Plate counts	2425	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts	2.0 ± 0.3 x 10^4	≥10^4 cfu ml^{-1}
Coliform bacteria	Bacterial counts	1.0 ± 1.0 x 10^0	-
Vibrio sp.	Bacterial counts	2.2 ± 0.6 x 10^4	-
Vibrio cholerae	Bacterial counts, elimination method	-	-
Enterococcus group	Bacterial counts	1.6 ± 0.4 x 10^1	-
Test 4			
Organisms ≥50 µm	Microscope counts	181050	≥10^5 m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method	1700	≥1000 ml^{-1}
	95 % conf. interval	700-4800	
	Microscope counts	1660	
	Plate counts	1750	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts	5.0 ± 0.4 x 10^4	≥10^4 cfu ml^{-1}
Coliform bacteria	Bacterial counts	0 ± 0	-
Vibrio sp.	Bacterial counts	2.9 ± 0.9 x 10^4	-
Vibrio cholerae	Bacterial counts, elimination method	-	-
Enterococcus group	Bacterial counts	6.3 ± 6.0 x 10^1	-

Test organism	Method	Influent	Requirement
Test 5			
Organisms ≥50 µm	Microscope counts	196467	$\geq 10^5$ m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method	1300	≥1000 ml^{-1}
	95 % conf. interval	500-3900	
	Microscope counts	1634	
	Plate counts	1700	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts	$1.3 \pm 0.23 \times 10^4$	$\geq 10^4$ cfu ml^{-1}
Coliform bacteria	Bacterial counts	$1.07 \pm 0.5 \times 10^2$	-
Vibrio sp.	Bacterial counts	$3.8 \pm 0.14 \times 10^3$	-
Vibrio cholerae	Bacterial counts, elimination method	-	-
Enterococcus group	Bacterial counts	$4.5 \pm 0.3 \times 10^2$	-
Test 6			
Organisms ≥50 µm	Microscope counts	189683	$\geq 10^5$ m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method	1700	≥1000 ml^{-1}
	95 % conf. interval	700-4800	
	Microscope counts	1736	
	Plate counts	1600	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts	$1.2 \pm 0.5 \times 10^5$	$\geq 10^4$ cfu ml^{-1}
Coliform bacteria	Bacterial counts	$5.0 \pm 4.0 \times 10^0$	-
Vibrio sp.	Bacterial counts	$1.8 \pm 0.5 \times 10^4$	-
Vibrio cholerae	Bacterial counts, elimination method	-	-
Enterococcus group	Bacterial counts	$8.0 \pm 0 \times 10^2$	-
Test 7			
Organisms ≥50 µm	Microscope counts	130038	$\geq 10^5$ m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method	2400	≥1000 ml^{-1}
	95 % conf. interval	1000-9500	
	Microscope counts	2023	
	Plate counts	750**	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts	$3.6 \pm 0.9 \times 10^4$	$\geq 10^4$ cfu ml^{-1}
Coliform bacteria	Bacterial counts	$4.4 \pm 0.7 \times 10^2$	-
Vibrio sp.	Bacterial counts	$8.9 \pm 0.9 \times 10^3$	-
Vibrio cholerae	Bacterial counts, elimination method	-	-
Enterococcus group	Bacterial counts	$4.2 \pm 0.1 \times 10^2$	-

Test organism	Method	Influent	Requirement
Test 8			
Organisms ≥50 µm	Microscope counts	197775	$\geq 10^5$ m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method	3000	≥1000 ml^{-1}
	95 % conf. interval	1000-13000	
	Microscope counts	1547 ± 54	
	Plate counts	1150	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts	$2.2 \pm 0.6 \times 10^4$	$\geq 10^4$ cfu ml^{-1}
Coliform bacteria	Bacterial counts	$3.0 \pm 2.0 \times 10^1$	-
Vibrio sp.	Bacterial counts	$6.0 \pm 1.7 \times 10^3$	-
Vibrio cholerae	Bacterial counts, elimination method	-	-
Enterococcus group	Bacterial counts	$7.4 \pm 1.0 \times 10^2$	-
Test 9			
Organisms ≥50 µm	Microscope counts	242817	$\geq 10^5$ m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method	2400	≥1000 ml^{-1}
	95 % conf. interval	1000-9500	
	Microscope counts	1382 ± 196	
	Plate counts	1725	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts	$1.1 \pm 0.03 \times 10^5$	$\geq 10^4$ cfu ml^{-1}
Coliform bacteria	Bacterial counts	$1.0 \pm 0 \times 10^0$	-
Vibrio sp.	Bacterial counts	$2.1 \pm 0.2 \times 10^3$	-
Vibrio cholerae	Bacterial counts, elimination method	-	-
Enterococcus group	Bacterial counts	$4.5 \pm 0.6 \times 10^2$	-
Test 10			
Organisms ≥50 µm	Microscope counts	202263	$\geq 10^5$ m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method	3000	≥1000 ml^{-1}
	95 % conf. interval	1000-13000	
	Microscope counts	1849 ± 422	
	Plate counts	1700	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts	$7.9 \pm 0.8 \times 10^4$	$\geq 10^4$ cfu ml^{-1}
Coliform bacteria	Bacterial counts	0 ± 1	-
Vibrio sp.	Bacterial counts	$1.1 \pm 0.7 \times 10^5$	-
Vibrio cholerae	Bacterial counts, elimination method	-	-
Enterococcus group	Bacterial counts	$1.3 \pm 0.6 \times 10^2$	-

Test organism	Method	Influent	Requirement
Test 11			
Organisms ≥50 µm	Microscope counts	210125	$\geq 10^5$ m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method	5000	≥1000 ml^{-1}
	95 % conf. interval	2000-20000	
	Microscope counts	1728	
	Plate counts	1850	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts	$4.5 \pm 0.5 \times 10^4$	$\geq 10^4$ cfu ml^{-1}
Coliform bacteria	Bacterial counts	0 ± 0	-
Vibrio sp.	Bacterial counts	$1.7 \pm 0.1 \times 10^3$	-
Vibrio cholerae	Bacterial counts, elimination method	-	-
Enterococcus group	Bacterial counts	$4.8 \pm 0.6 \times 10^1$	-
Test 12			
Organisms ≥50 µm	Microscope counts	171213	$\geq 10^5$ m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method	3000	≥1000 ml^{-1}
	95 % conf. interval	1000-13000	
	Microscope counts	2039	
	Plate counts	1350	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts	$3.0 \pm 1.0 \times 10^4$	$\geq 10^4$ cfu ml^{-1}
Coliform bacteria	Bacterial counts	0 ± 0	-
Vibrio sp.	Bacterial counts	$5.0 \pm 1.7 \times 10^3$	-
Vibrio cholerae	Bacterial counts, elimination method	-	-
Enterococcus group	Bacterial counts	$9.9 \pm 1.0 \times 10^1$	-
Test 13			
Organisms ≥50 µm	Microscope counts	114246	$\geq 10^5$ m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method	1300	≥1000 ml^{-1}
	95 % conf. interval	500-3900	
	Microscope counts	1331	
	Plate counts	1500	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts	$2.1 \pm 0.2 \times 10^4$	$\geq 10^4$ cfu ml^{-1}
Coliform bacteria	Bacterial counts	0 ± 0	-
Vibrio sp.	Bacterial counts	$2.2 \pm 2.6 \times 10^3$	-
Vibrio cholerae	Bacterial counts, elimination method	-	-
Enterococcus group	Bacterial counts	$9.0 \pm 0.9 \times 10^1$	-

*While this sample indicate value below 1000/ml, sample from CT1 from same date gave 2000 cells/ml. Also plate counts and microscope counts gave >1000 cells/ml. It should be taken into account that culture methods normally underestimate the true number of algae present in the sample due to the fact that not all species will grow on laboratory media. ** Plate count method is only used to give a preliminary indication of the algae density in the sample and is therefore not conclusive.

3.6 Biocidal effects on organisms ≥50 µm in minimum diameter

The number of viable organisms ≥50 µm in minimum diameter, as determined on the basis of motility and integrity by microcope examination in treated test water and control immediately after treatment and after five days of storage, is given in **Table 14.** Values in brackets are recounts of samples after 24 h, and should be used in the assessment of the technology, because it takes some time before full effect of the treatment is achieved for artemia. Allthough the challenge water included many species in the >50 µm group, only artemia was ever found as viable organism in samples after treatment. Numbers of organisms ≥ 50 um measured on day 5, where sometimes higher than measurements made on day 0, and for test cycle 1 and 2, the numbers of organisms did not meet the requirements as set in regulation D-2 of the IMO Guidelines (< 10 organisms /m^3) and as shown in **Table 2**. The reason for this was residual non treated challenge water containing artemia in a segment of the outside piping of the test facility to the tanks. Better cleaning procedures and flushing of the pipeline reduced this problem for test cycles 3 to 9. New piping installed before test cycle 10 eliminated this problem.

The equivalent requirements for the non treated water (control) is stated as: " If in any test the average discharge results from the control water is a concentration less then or equal to 10 times the values in regulation D-2.1, the test cycle is invalid." This has been interpreted to that the minimum level of viable organisms in the control water at the time of discharge (e.g after 5 days storage) should be higher than 100 organisms per m^3. This requirement was fulfilled in all tests.

Table 14 Viable organisms ≥50 µm in minimum diameter in treated test water and control immediately after treatment and after five days of storage. Green background indicates that required level was fulfilled, yellow background partial fulfilment, while red background indicates failure to fulfil required level. (a.d. = after deballasting on day 5).

	Treated water		**Control water**	
	Day 0	**Day 5 (a.d)**	**Day 0**	**Day 5 (a.d.)**
Organisms ≥50 µm in minimum diameter (individuals m^{-3})				
Requirement	-	<10	-	>100
Test cycle 1	1.0 ± 1.0 (0.3)	28 ± 16.5 (3.3)*	28444 ± 2350	5531 ± 1878
Test cycle 2	0 ± 0	19 ± 11.3 (4)*	28137 ± 12816	11693 ± 4476
Test cycle 3	0 ± 0	8.3 ± 3.2 (4.3)	20767 ± 12915	5338 ± 1571
Test cycle 4	0.3 ± 0.6 (0.3)	1.0 ± 1.0 (0.3)	39985 ± 13102	6934 ± 1976
Test cycle 5	0 ± 0	6.3 ± 3.1 (3.3)	24424 ± 3044	10356 ± 1901
Test cycle 6	63.7 ± 33.2 (4.7)	3.3 ± 0.6 (1.7)	51785 ± 21654	7157 ± 3099
Test cycle 7	1.0 ± 1.0 (0.7)	2.7 ± 1.5 (1.0)	54498 ± 10229	12411 ± 1977
Test cycle 8	20 ± 8.2 (1.7)	2.3 ± 1.3 (2.3)	36865 ± 8462	4586 ± 4586
Test cycle 9	51.3 ± 17 (10.3)	6.3 ± 3.8 (4.0)	59852 ± 11827	20931 ± 2778
Test cycle 10	8.7 ± 3.5 (2.0)	1.7 ± 1.2 (0.7)	42648 ± 12974	25635 ± 3414
Test cycle 11	0 ± 0	1.7 ± 0.6 (1.0)	31361 ±12233	10256 ±1650
Test cycle 12	0 ± 0	0.3 ± 0.6 (0.3)	31115 ± 5049	8608 ± 3216
Test cycle 13	0 ± 0	1.3 ± 0.6 (1.0)	22322 ± 254	13665 ± 6950

* Caused by contamination trapped in the pipes of the water transfer and treatment system being caught in a space which was not sufficiently flushed upon commencing the deballasting operation.

3.7 Biocidal effects on organisms ≥10-50 µm in minimum diameter

The number of viable organisms' ≥10-50 µm in minimum diameter, as determined by the serial dilution method in algal growth medium, by microscopy examination after incubation with CFDA-AM and plating on seawater agar in treated test water and control immediately after treatment and

after five days of storage, is given in **Table 15**. For the performance of the ballast water treatment system to pass the regulation D-2 of the IMO guidelines, as shown in **Table 2,** less then 10 viable organisms per ml should be present in the treated water after five days storage. This requirement was fulfilled in all test cycles using all three detection methods, of these; the microscope count is the most accurate one. In the full seawater test, surving species ≥10 µm were most commonly coccolithophorids (probably *Emiliania huxleyii)*, *Cheatoceros* sp. or dinoflagellates, in that order of importance. When testing brackish water *T. Sueica* where sometimes seen survive on day 0, but never after day 5 treatment. Again Coccolithophorids were the main surviving species in treated samples. *Emiliania huxleyii* does not stain with CFDA very well and the microscope method therefore underestimates this group relative to the serial dilution method.

The equivalent requirements for the non treated water (control) is stated as: " If in any test the average discharge results from the control water is a concentration less then or equal to 10 times the values in regulation D-2.1, the test cycle is invalid." This has been interpreted to that the minimum level of viable organisms in the control water at the time of discharge (e.g after 5 days storage) should be higher than 100 organisms per ml. This requirement was fulfilled in all tests.

Table 15. Viable organisms ≥10-50 µm in minimum diameter in treated test water and control immediately after treatment and after five days of storage. Green background indicates that required level was fulfilled, yellow background partial fulfilment, while red background indicates failure to fulfil required level. (a.d.= after deballasting on day 5)

	Treated water		**Control water**	
	Day 0	**Day 5 (a.d.)**	**Day 0**	**Day 5 (a.d.)**
Organisms ≥10-50 µm in minimum diameter (individuals ml^{-1})				
Requirement	-	<10	-	>100
Dilution method (95 % confidence interval)				
Test cycle 1	0.4	<0.2	9000	2400
	<0.1-1.7	<0.1-1.0	3000-39000	1000-9500
Test cycle 2	13	<0.2	2000	2400
	0.5-3.9	<0.1-1.0	1000-14000	1000-9500
Test cycle 3	<0.2	0.2	2000	2400
	<0.1-1.0	0.1-1.1	1000-14000	1000-9500
Test cycle 4	<0.2	<0.2	2000	2400
	<0.1-1.0	<0.1-1.0	1000-14000	1000-9500
Test cycle 5	1.1	<0.2	1500	2400
	0.4-2.9	<0.1-1.0	500-5100	1000-9500
Test cycle 6	5	0.2	1500	2400
	2-17	<0.1-1.1	500-5100	1000-9500
Test cycle 7	13	1.3	2000	1300
	5-39	0.5-3.9	1000-14000	500-3900
Test cycle 8	5	5.9	2000	2400
	2-15	2.4-20	1000-14000	1000-9500
Test cycle 9	160	8.8	2000	1300
	60-530	3.3-28	1000-14000	500-3900
Test cycle 10	31	5.4	5000	800
	14.5-77	2 – 19.2	2000-24000	300 - 2500
Test cycle 11	12.2	1.7	5000	2400
	5 - 40	0.8 – 4.1	2000-24000	1000-9500
Test cycle 12	3.1	1.7	7000	2400
	1.4 – 8.3	0.7 – 4.6	2000-28000	1000-9500
Test cycle 13	13	0.4	11000	1300
	5 - 39	0.1 – 1.7	3000-48000	500 - 3900

	Treated water		Control water	
	Day 0	Day 5 (a.d.)	Day 0	Day 5 (a.d.)
Microscope counts				
Test cycle 1	0 ± 0	0.7 ± 0.6	2316 ± 447	2800 ± 259
Test cycle 2	0 ± 0	0 ± 0	1487 ± 196	2074 ± 452
Test cycle 3	0.3 ± 0.6	0.3 ± 0.6	2213 ± 131	2930 ± 349
Tect cycle 4	0 ± 0	0 ± 0	1694 ± 364	2005 ± 317
Test cycle 5	0 ± 0	0 ± 0	1634 ± 368	1988 ± 358
Test cycle 6	1.7 ± 1.5	0.3 ± 0.6	1867 ± 340	1746 ± 240
Test cycle 7	2.7 ± 1.5	1.0 ± 1.0	1642 ± 490	1539 ± 393
Test cycle 8	3.7 ± 0.6	1.0 ± 1.0	1901 ± 118	1633 ± 182
Test cycle 9	169 ± 47	1.7 ± 0.6	1780 ± 108	1694 ± 120
Test cycle 10	28.7 ± 8.3	0 ± 0	1763 ± 187	1607 ± 104
Test cycle 11	0 ± 0	0.7 ± 0.6	1624 ± 130	1452 ± 52
Test cycle 12	0 ± 0	0 ± 0	1763 ± 274	1573 ± 104
Test cycle 13	0 ± 0	0 ± 0	1642 ± 130	1521 ± 266
Plate counts				
Test cycle 1	<10	<10	-	1500
Test cycle 2	<10	<10	-	1250
Test cycle 3	<10	<10	-	1600
Test cycle 4	<10	<10	-	1370
Test cycle 5	<10	<10	-	1120
Test cycle 6	<10	<10	-	900
Test cycle 7	<10	<10	-	1050
Test cycle 8	<10	<10	-	1200
Test cycle 9	57	<10	-	560
Test cycle 10	<10	<10	-	550
Test cycle 11	<10	<10	-	1850
Test cycle 12	<10	<10	-	910
Test cycle 13	<10	<10	-	950

3.8 Bactericidal effects

The numbers of heterotrophic bacteria, coliform bacteria, *E.coli, Vibrio* sp., *Vibrio cholerae, Enterococcus* group and intestinal *Enterococci*, determined in treated water and control water immediately after treatment and after five days of storage are given in **Table 16**. Regulation D-2 only requires documentation of the bactericidal effect of the ballast water treatment system on *E.coli*, *Vibrio cholera* (toxicogenic serotypes O1 and 0139) and intestinal *Enterococi* given as maximum allowable concentration in discharge waters of < 250 cfu/ml, < 1 cfu/ml and < 100 cfu/ml, respectively. These requirements were fulfilled for all bacterial species in all test cycles. *Vibrio* sp. is common in salt and brackish surface water. However, the possibility of further detection of the serotypes O1 and O139 is very small or absent since none of these serotypes have been isolated from Norwegian coastal water. *Vibrio cholera* was not found in any of the samples from treated water during the 13 test cycles.

The treatment system reduced the numbers of heterotrophic bacteria and *Vibrio* sp. with approximately 3-4 log units, during ballasting operation with seawater. During brackish water test, the treatment efficiency during ballasting operation was reduced to 1-3 log units. This is probably due to a more rapid decay and thus reduced efficiency of disinfectionous substances (i.e. chlorine) in brackish water compared to seawater. Coliforms and enterococcus bacteria was present in the test water in concentrations up to 10^2 cfu/100 ml, but could hardly be detected (< 1 cfu/100 ml) in treated water after treatment (ballasting - day 0) or after 5 days storage and treatment (deballasting – day 5).

Table 16. Heterotrophic bactera, coliform bacteria, *E.coli, Vibrio* sp. *Vibrio cholera*, Enterococcus group and intestinal *Enterococci* in treated test water and control water immediately after treatment and after five days of storage for test cycles 1-13. Green background indicates that required level was fulfilled, yellow background partial fulfilment, while red background indicates failure to fulfil required level. (a.d.= after deballasting on day 5).

	Treated water			**Control water**	
	Day 0	**Day 5 (a.d.)**		**Day 0**	**Day 5 (a.d.)**
Marine heterotrophic bacteria (cfu ml^{-1})					
Requirement	-	-		-	-
Test cycle 1	$8.4 \pm 2.3 \times 10^{0}$	$6.9 \pm 4.4 \times 10^{0}$		$3.7 \pm 0.6 \times 10^{4}$	$6.6 \pm 0.5 \times 10^{4}$
Test cycle 2	$1.5 \pm 1.0 \times 10^{0}$	$0.9 \pm 0.9 \times 10^{0}$		$1.7 \pm 0.9 \times 10^{5}$	$5.2 \pm 0.8 \times 10^{4}$
Test cycle 3	$6.4 \pm 5.8 \times 10^{1}$	$1.0 \pm 0.8 \times 10^{1}$		$3.2 \pm 1.4 \times 10^{4}$	$9.7 \pm 1.9 \times 10^{4}$
Test cycle 4	$4.8 \pm 3.8 \times 10^{0}$	$6.0 \pm 3.6 \times 10^{0}$		$5.8 \pm 1.7 \times 10^{4}$	$4.3 \pm 1.4 \times 10^{5}$
Test cycle 5	$1.1 \pm 1.9 \times 10^{1}$	$1.3 \pm 0.6 \times 10^{0}$		$1.3 \pm 1.1 \times 10^{4}$	$1.1 \pm 0.2 \times 10^{5}$
Test cycle 6	$7.0 \pm 1.0 \times 10^{2}$	$9.3 \pm 8.1 \times 10^{0}$		$1.6 \pm 0.3 \times 10^{5}$	$1.6 \pm 0.2 \times 10^{4}$
Test cycle 7	$1.1 \pm 0.3 \times 10^{2}$	$6.5 \pm 2.6 \times 10^{1}$		$4.3 \pm 1.2 \times 10^{4}$	$5.3 \pm 2.9 \times 10^{4}$
Test cycle 8	$2.1 \pm 0.5 \times 10^{2}$	$5.7 \pm 0.3 \times 10^{1}$		$2.7 \pm 0.4 \times 10^{4}$	$5.4 \pm 3.9 \times 10^{4}$
Test cycle 9	$2.2 \pm 1.5 \times 10^{3}$	$6.9 \pm 1.4 \times 10^{0}$		$1.8 \pm 0.3 \times 10^{5}$	$3.4 \pm 0.6 \times 10^{4}$
Test cycle 10	$3.9 \pm 1.1 \times 10^{2}$	$1.4 \pm 0.2 \times 10^{2}$		$1.1 \pm 0.1 \times 10^{5}$	$3.5 \pm 1.0 \times 10^{4}$
Test cycle 11	$9.3 \pm 2.9 \times 10^{0}$	$3.8 \pm 2.6 \times 10^{2}$		$5.9 \pm 1.6 \times 10^{4}$	$2.5 \pm 0.7 \times 10^{4}$
Test cycle 12	$1.8 \pm 0.9 \times 10^{0}$	$3.0 \pm 1.7 \times 10^{1}$		$3.3 \pm 0.3 \times 10^{4}$	$1.6 \pm 0.1 \times 10^{5}$
Test cycle 13	$5.4 \pm 5.6 \times 10^{0}$	$2.7 \pm 4.7 \times 10^{0}$		$2.2 \pm 0.2 \times 10^{4}$	$2.2 \pm 0.2 \times 10^{4}$
Coliform bacteria (Coli.) and *Escherichia coli (E. coli) (cfu 100 ml^{-1})**					
	Coli.	**Coli.**	***E. coli***	**Coli.**	**Coli.**
Requirement	-	-	<250*	-	-
Test cycle 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Test cycle 2	0 ± 0	0.3 ± 0.6	0 ± 0	0 ± 0	0 ± 0
Test cycle 3	0 ± 1	0 ± 0	0 ± 0	$2.7 \pm 0.6 \times 10^{1}$	$1.4 \pm 0.7 \times 10^{2}$
Test cycle 4	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Test cycle 5	0 ± 0	0 ± 0	0 ± 0	$2.0 \pm 0.3 \times 10^{2}$	1 ± 1
Test cycle 6	0 ± 0	0 ± 0	0 ± 0	1.0 ± 1.0	0 ± 0
Test cycle 7	0 ± 0	0 ± 0	0 ± 0	$4.5 \pm 2.6 \times 10^{2}$	$1.3 \pm 1.2 \times 10^{1}$
Test cycle 8	0 ± 0	0 ± 0	0 ± 0	$1.3 \pm 2.3 \times 10^{1}$	0 ± 0
Test cycle 9	0 ± 0	0 ± 0	0 ± 0	2.0 ± 1.0	0 ± 0
Test cycle 10	0 ± 0	0 ± 0	0 ± 0	1.0 ± 1.0	0 ± 0
Test cycle 11	0 ± 0	0 ± 0	0 ± 0	0 ± 1	0 ± 0
Test cycle 12	1.0 ± 1.0	0 ± 0	0 ± 0	1.0 ± 1.0	0 ± 0
Test cycle 13	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

	Treated water			Control water	
	Day 0	**Day 5 (a.d.)**		**Day 0**	**Day 5 (a.d.)**
Vibrio* sp. and *Vibrio cholerae (*V. cholerae*) (cfu 100 ml^{-1})**					
	***Vibrio* sp.**	***Vibrio* sp**	***V. cholerae***	***Vibrio* sp.**	***Vibrio* sp.**
Requirement	-	-	<1**	-	-
Test cycle 1	$5.5 \pm 4.8 \times 10^{0}$	$0.6 \pm 0.5 \times 10^{0}$	< 1 **	$1.7 \pm 0.6 \times 10^{4}$	$5.9 \pm 1.1 \times 10^{3}$
Test cycle 2	$2.4 \pm 1.4 \times 10^{0}$	$0.6 \pm 1.0 \times 10^{0}$	< 1 **	$7.4 \pm 0.5 \times 10^{4}$	$1.7 \pm 0.2 \times 10^{4}$
Test cycle 3	$9.9 \pm 0.0 \times 10^{0}$	$0.3 \pm 0.5 \times 10^{0}$	< 1 **	$1.9 \pm 0.8 \times 10^{4}$	$2.4 \pm 0.2 \times 10^{4}$
Test cycle 4	$4.5 \pm 1.6 \times 10^{0}$	0 ± 0	< 1 **	$3.4 \pm 1.3 \times 10^{4}$	$1.9 \pm 0.2 \times 10^{4}$
Test cycle 5	$1.0 \pm 1.6 \times 10^{0}$	0 ± 0	< 1 **	$3.0 \pm 2.6 \times 10^{3}$	$2.5 \pm 0.3 \times 10^{3}$
Test cycle 6	$1.3 \pm 0.2 \times 10^{2}$	$0.6 \pm 1.0 \times 10^{0}$	< 1**	$1.0 \pm 0.5 \times 10^{4}$	$3.3 \pm 0.2 \times 10^{3}$
Test cycle 7	$1.1 \pm 0.2 \times 10^{2}$	$3.2 \pm 0.2 \times 10^{1}$	< 1 **	$9.5 \pm 0.3 \times 10^{3}$	$1.8 \pm 0.2 \times 10^{3}$
Test cycle 8	$2.5 \pm 0.4 \times 10^{2}$	$3.4 \pm 1.3 \times 10^{1}$	< 1 **	$8.0 \pm 2.5 \times 10^{3}$	$2.1 \pm 0.05 \times 10^{3}$
Test cycle 9	$1.0 \pm 0.0 \times 10^{2}$	$1.8 \pm 1.6 \times 10^{0}$	< 1 **	$2.9 \pm 1.2 \times 10^{4}$	$2.5 \pm 0.3 \times 10^{3}$
Test cycle 10	$2.6 \pm 0.6 \times 10^{2}$	$2.1 \pm 1.4 \times 10^{0}$	< 1 **	$4.6 \pm 0.6 \times 10^{4}$	$1.1 \pm 0.2 \times 10^{4}$
Test cycle 11	$1.0 \pm 1.0 \times 10^{0}$	$1.4 \pm 2.0 \times 10^{1}$	< 1 **	$3.8 \pm 0.8 \times 10^{3}$	$9.7 \pm 1.2 \times 10^{2}$
Test cycle 12	$1.2 \pm 0.5 \times 10^{0}$	$0.6 \pm 1.0 \times 10^{0}$	< 1 **	$1.2 \pm 0.3 \times 10^{4}$	$2.4 \pm 0.6 \times 10^{3}$
Test cycle 13	$3.3 \pm 0.5 \times 10^{0}$	0 ± 0	< 1 **	$4.2 \pm 0.2 \times 10^{3}$	$9.1 \pm 1.3 \times 10^{2}$
Enterococcus group (Ent. gr.) and Intestinal *Enterococci* (Int. *Ent.*)* (cfu 100 ml^{-1})**					
	Ent. gr.	**Ent. gr.**	**Int. *Ent.***	**Ent. gr.**	**Ent. gr.**
Requirement	-	-	<100***	-	-
Test cycle 1	0 ± 0	0 ± 0	0 ± 0	$1.7 \pm 0.6 \times 10^{0}$	0 ± 0
Test cycle 2	0 ± 0	$3.0 \pm 2.0 \times 10^{0}$	0 ± 0	$1.0 \pm 0 \times 10^{2}$	$1.8 \pm 0.4 \times 10^{1}$
Test cycle 3	0 ± 0	0 ± 1	0 ± 0	$1.5 \pm 0 \times 10^{1}$	$4.0 \pm 2.0 \times 10^{0}$
Test cycle 4	$2.0 \pm 2.0 \times 10^{0}$	0 ± 0	0 ± 0	$8.0 \pm 6.0 \times 10^{0}$	$6.0 \pm 1.0 \times 10^{0}$
Test cycle 5	0 ± 0	0 ± 0	0 ± 0	$3.4 \pm 0.1 \times 10^{2}$	$1.4 \pm 0.3 \times 10^{2}$
Test cycle 6	0 ± 0	0 ± 0	0 ± 0	$8.0 \pm 0 \times 10^{2}$	$1.8 \pm 0.5 \times 10^{1}$
Test cycle 7	0 ± 0	0 ± 0	0 ± 0	$4.0 \pm 0.7 \times 10^{2}$	$5.0 \pm 1.0 \times 10^{0}$
Test cycle 8	1 ± 1	$3.0 \pm 2.0 \times 10^{0}$	0 ± 0	$1.0 \pm 0.08 \times 10^{3}$	$2.9 \pm 0.3 \times 10^{1}$
Test cycle 9	$1.0 \pm 2.0 \times 10^{0}$	$2.0 \pm 3.0 \times 10^{0}$	0 ± 0	$4.0 \pm 1.0 \times 10^{0}$	$2.0 \pm 2.0 \times 10^{0}$
Test cycle 10	$1.0 \pm 1.0 \times 10^{0}$	0 ± 0	0 ± 0	$1.6 \pm 0 \times 10^{2}$	0 ± 0
Test cycle 11	0 ± 0	0 ± 0	0 ± 0	$4.6 \pm 1.0 \times 10^{1}$	0 ± 1
Test cycle 12	1.0 ± 1.0	0 ± 0	0 ± 0	$1.0 \pm 0.05 \times 10^{2}$	$2.3 \pm 0.1 \times 10^{1}$
Test cycle 13	0 ± 0	0 ± 0	0 ± 0	$9.5 \pm 0.4 \times 10^{1}$	$0.6 \pm 0.3 \times 10^{1}$

* The figures refer to the number identified as *Escherichia coli*/100 ml within the group of coliform bacteria. There is a requirement for *Escherichia coli* being <250 cfu/100ml after five days storage (D-2).
** The figures refer to the number identified as *Vibrio cholerae*/100 ml after five days storage. There is a requirement for toxicogenic *Vibrio cholerae* (serotypes O1 and O139) being <1 cfu/100 ml after five days storage (D-2).
*** The figures refer to the number identified as intestinal *Enterococci*/100 ml within the group of Enterococcus. There is a requirement for intestinal *Enterococci* being <100 cfu/100ml after five days storage (D-2).

3.9 Ecotoxicological responses

The IMO regulations require that treated ballast water as a minimum should be toxicity tested both acutely and chronically with multiple test species (a fish, an invertebrate and a plant). As control, non treated ballast water has been used to ensure that only the effect of the treatment system is tested. The tests and results are summarized below. The complete test reports are presented in **Appendix K** – Toxicity tests.

Fish toxicity testing

Table 17 summarize the results of the fish toxicity tests with turbot (*Scopthalmus maximus*) performed with treated ballast water. In the acute tests mortality was only observed in full seawater (test cycle 1 and 2). It is the results from test cycle 2 that should be used for risk assessment purposes as test cycle 1 was not considered typical of a normal treatment cycle. No acute effects were observed when testing treated brackish water.

Chronic toxicity tests with turbot (juvenile growth test) have also been performed in treated seawater and brackish water. Both gave a NOEC of 56 %. This may seem a bit surprising as the fish died at 100 % in full seawater while none died at 100 % in brackish water. However the effect was quite clear with a 50 % reduction in growth in fish exposed to 100 % relative to control fish.

Table 17. Summary of the results from toxicity testing of ballast water treated with the CleanBallast treatment system at day 5 after deballasting using juvenile turbot

Fish Acute tests				
Test cycle	**Test id**	**Test sample**	**Result LC50 (%)**	**Comment**
1	Acute turbot	08.09.08	42	Not relevant for use in risk assessment
2	Acute turbot	15.09.08	74	
6	Acute turbot	07.10.08	> 100 %	
11	Acute turbot	02.11.08	> 100 %	
Fish Chronic test				
Test cycle	**Test id**	**Test sample**	**Result NOEC (%)**	**Comment**
2-5	Chronic turbot	15.09.08-02.10.08	56 %	
6-10	Chronic turbot	07.10.08-27.10.08	56 %	

Invertebrate toxicity testing

The test results of the invertebrate testing are presented in **Table 18**. Acute tests with *Acartia tonsa*, showed that this species is the least sensitive with respect to mortality. The chronic studies with *Nitocra spinipes*, showed no effect at the highest tested concentration of 32 % when testing brackish water. Therefore the NOEC here was ≥32 %. For full seawater the first test had a general low reproduction in both control and test water. The test was repeated during during test cycle 12 and 13. The test concentration used was 32 % Results showed that the NOEC was > 32%.

Table 18. Test results of toxicity testing of ballast water treated with the CleanBallast treatment system at day 5 after deballasting with invertebrates.

Acute Invertebrate tests				
Test Cycle	**Test id**	**Test sample**	**Result LC50 %**	**Comment**
1	*Acartia tonsa* acute	08.09.2008	60.5 %	Full seawater
7	*Acartia tonsa* acute	12.10.08	> 100 %	Brackish water
11	*Acartia tonsa* acute	02.11.08	> 100 %	Full seawater
Chronic Invertebrate tests				
Test Cycle	**Test id**	**Test sample**	**Result NOEC %**	**Comment**
2-3	*Nitocra* Reproduction	17.09.08-22.09.08	> 18 %	Highest tested concentration was 18 %, test was repeated with test cycle 12-13 water
9-10	*Nitocra* Reproduction	22.10.08-27.10.08	>32 %	Highest tested concentration was 32 %
12-13	*Nitocra* Reproduction	6.11.08-21.11.08	>32 %	Highest tested concentration was 32 %

Growth inhibition of the marine alga *Skeletonema costatum*

Growth inhibition tests with the marine alga *Skeletonema costatum* has been performed on samples of treated ballast water from all test cycles. The effect concentrations (EC_{10} and EC_{50}) are expressed as percentage of treated ballast water diluted that causes a specific reduction of the growth rate of the algae as compared to the growth in untreated ballast water (control). Thus, EC_{10} is the concentration of ballast water in which the growth rate of *S. costatum* is reduced by 10 % and EC_{50} the concentration at which the growth rate reduction is 50 %. Consequently, a low effect concentration (e.g. EC_{50}) indicates high toxicity. Results are shown in **Table 19.**

The effect concentrations showed generally higher toxicity in test cycles with seawater than in brackish water. In the seawater tests, the EC_{10} varied from 14 to 56 % (average 33 %) and EC_{50} from 19 to 78 % (average 44 %) when excluding test 11, day 5. In test cycle 11, day 5, the TRO was neutralized by addition of sodium thiosulphate and EC_{10} and EC_{50} were both >100%, showing that the neutralization step efficiently removed the toxicity.

At all test cycles with seawater, the algae growth inhibition tests showed higher toxicity on day 5 than on day 0. The average EC_{50} values were 57 % on day 0 and 30 % on day 5.

All tests performed in brackish water showed a low toxicity of the treated ballast water. EC_{10} could only be calculated in four of the ten tests conducted. The treated ballast water gave less than 50% growth reduction in all tests and, hence, no EC_{50} values could be determined.

Table 19. Effect concentrations obtained in growth inhibition tests of treated ballast water with the algae *S. costatum*.

Test cycle	Day nr	Date	EC10 (%)	EC50 (%)
1	0	03.09.2008	30	40
1	5	08.09.2008	14	19
2	0	10.09.2008	25	34
2	5	15.09.2008	17	20
3	0	17.09.2008	>32	> 32
3	5	22.09.2008	14	19
4	0	22.09.2008	45	64
4	5	27.09.2008	17	21
5	0	27.09.2008	40	78
5	5	02.10.2008	50	56
6	0	02.10.2008	73	> 100
6	5	07.10.2008	>100	> 100
7	0	07.10.2008	>100	> 100
7	5	12.10.2008	71	> 100
8	0	12.10.2008	61	> 100
8	5	17.10.2008	81	> 100
9	0	17.10.2008	>100	> 100
9	5	22.10.2008	>100	> 100
10	0	22.10.2008	>100	> 100
10	5	27.10.2008	>100	> 100
11	0	27.10.2008	56	64
11	5	01.11.2008	>100	> 100
12	0	01.11.2008	54	63
12	5	06.11.2008	33	45
13	0	06.11.2008	50	59
13	5	11.11.2008	22	29

The lower toxicity in the brackish water tests as compared with the seawater tests coincides with lower concentrations of TRO in brackish water tests as shown in **Table 8**. Furthermore, the fact that no algal toxicity was found in ballast water after neutralization with sodium tiosulphate supports the hypothesis that the growth inhibiting effect was mainly caused by TRO. In **Figure 4** and **Figure** 5 the toxic response (EC_{10}) of the algae is plotted against the concentrations of free and total TRO for all tests with measurable effects (day 0 and day 5). The inserted linear regressions indicate a significant correlation.

The fact that toxicity was consistently higher at day 5 than at day 0 in the seawater tests is not reflected by the measurements of TRO. The average total TRO was the same on day 0 and day 5 at the time of manual analysis, and the free TRO was slightly lower on day 5 than on day 0. Unless this can be explained by systematic errors in the TRO measurements, it indicates that other factors than TRO may have contributed to the observed growth inhibiting effects. As measured byproducts are lower in treated seawater than in brackish water it is not believed that this explains the difference. One may hypothesise that the TRO changes the algal medium quality during the storage time by changing the availability of micronutrients (chealators, vitamins etc).

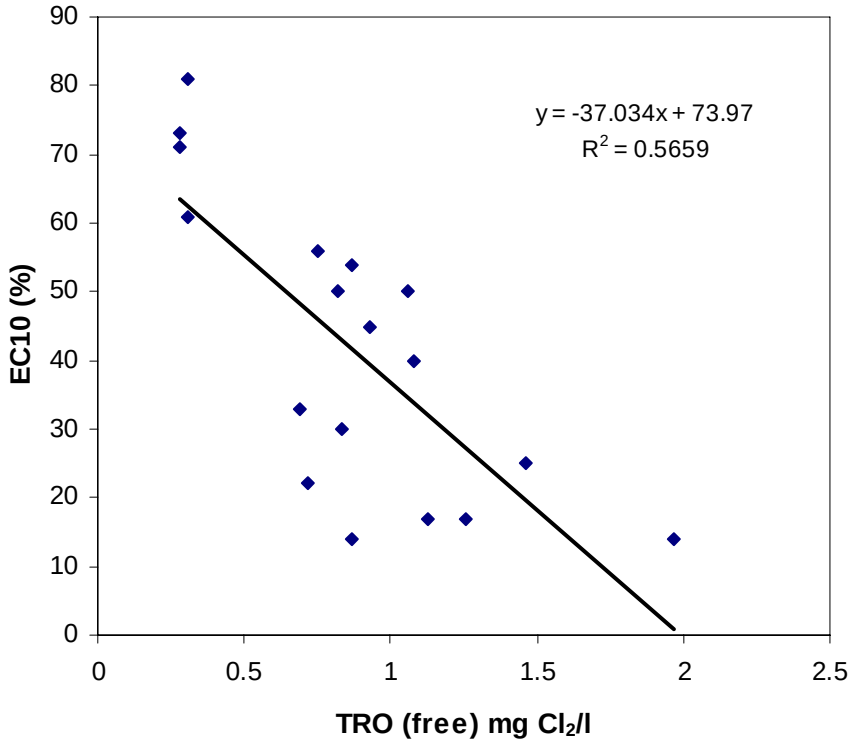

Figure 4. Toxic response of *S. costatum*, expressed as EC10 as a function of concentration of free TRO.

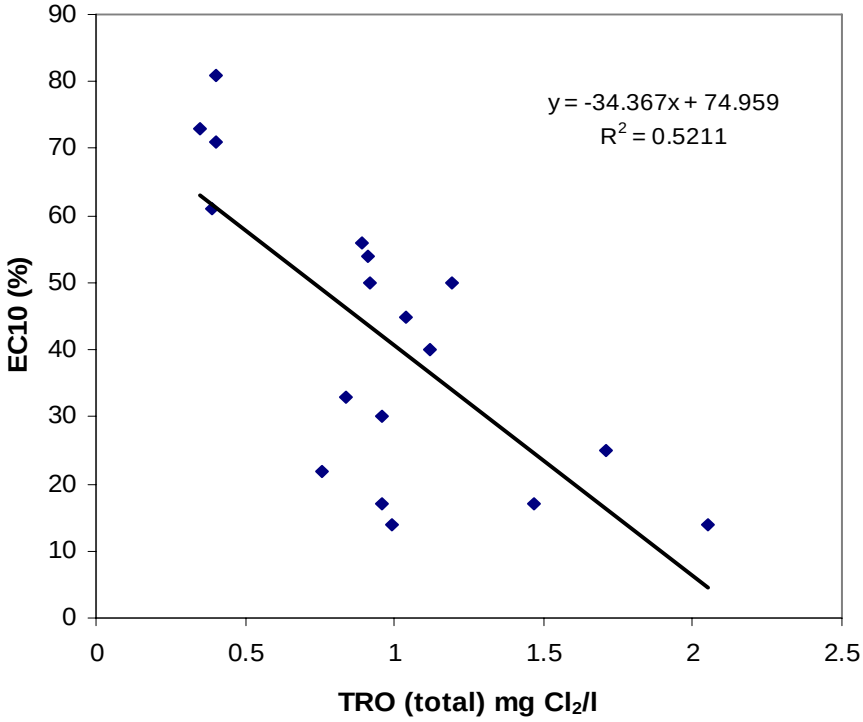

Figure 5. Toxic response of *S. costatum*, expressed as EC10 as a function of concentration of total TRO.

Toxicity decay studies
The decay of toxicity was studied on treated ballast water from test cycle 2, day 5 (seawater) and test cycle 9, day 0 (brackish water). Day 0 was chosen for studies due to an internal communication error. According to the QAPP, the studies should have been done on day 5 samples. However, since no effects were seen on either day 0 or day 5 samples, the decay of toxicity could not be measured. The results are shown in **Table 20** and **Table 21**.

The effect concentrations obtained in the decay study in seawater did not indicate a significant decline of toxicity in the period 5-52 hours after ballast water treatment. In the brackish water test, the effect concentrations EC_{10} and EC_{50} were >100% and consequently decay of toxicity can not be observed. The EC10 obtained after 16 hours indicates a small increase in toxicity but in this particular growth inhibition test the growth pattern deviated from the normal pattern by having a lag-phase during the first day which reduces the confidence of the test.

Table 20. Results of toxicity decay study in seawater (test cycle 2, day 5).

Time after treatment (hours)	EC10 (%)	EC50 (%)
5	17	20
20	17	26
29	22	30
52	19	26

Table 21. Results of toxicity decay study in brackish water (test cycle 9, day 0).

Time after treatment (hours)	EC10 (%)	EC50 (%)
3.5	>100	>100
16	77	>100
28	>100	>100

Oyster early life stage test
The toxicity of treated ballast water was tested on embryo of the oyster (*Crassostrea gigas*) following the ASTM E724 protocol: "Conducting static toxicity tests starting with embryos of four species of saltwater bivalve molluscs." Non-treated control ballast water was used as control. The results of the 3 tests are presented in **Table 22.** The results of testing of test cycle 5 gave a NOEC of 46 %, which was the next highest tested concentration. Comparing this with the EC_{10} of 50 % for algal test for the same test cycle indicate similar sensitivity for these two test systems.

As shown, the test with brackish water on test cycle 8 and test cycle 11 failed.
NIVA test personnel has prior experience with this method having done more than 50 of these tests and have not had this problem before. At the moment it is suspected that the adults that were received were not of the necessary quality, however, tests will be reviewed carefully in order to understand why this happened. Below is a description from the test executor on why the test failed.

Oyster embryos failed to develop into normal D-larvae in any of the treatment groups including the control. The reasons for this are likely to be two fold.

1. Due to the time of year oysters have a tendency to reabsorb their gametes resulting in poor egg and sperm quality. The same batch of conditioned oysters was distributed by Guernsey Sea Farms to two other laboratories including NIVA. For all laboratories test failure was reported.
2. The presence of bacteria in the control ballast water may also be having a detrimental effect on egg development.

The second explanation was thought since there were no normal D-larvae present in the controls. If gamete quality was the only factor a reduction in the proportion of normal D-larvae would be expected not 100% abnormality. For future tests, aged filtered seawater will be used as well as the control ballast water to reduce the possible impact of bacterial effects. It is expected for the quality of the conditioned oysters to improve with time.

Table 22. Test results of toxicity testing of ballast water treated with the CleanBallast treatment system at day 5 after deballasting using the oyster embryo test.

Chronic oyster embyo tests					
Test Cycle	**Test id**	**Test sample**	**Result LC50 %**	**Result NOEC (%)**	**Comment**
5	Chronic Oyster embryo	02.10.2008	62%	46	
8	Chronic Oyster embryo	17.10.08	none		No fertilization
11	Chronic Oyster embryo	02.11.08	none		No fertilization

Reproduction test with rotatoria *Brachionus plicatilis*

The test was conducted according to ISO draft guideline ISO/DC 20666-2008 "Water Quality – determination of the chronic toxicity to *Brachionus calyciflorus* in 48h". This is a test for a freshwater rotifer and the test protocol was adapted to the saltwater species *Brachionus plicatilis*. The main amendments when using *Brachionus plicatilis* as test organisms were: Use of seawater algal culture, test temperature of 20 ^{o}C instead of 25 ^{o}C and 96h test period instead of 48h. The longer test period was required because *B. plicatili* has a much lower growth rate than *B. calyciflorus*. The results are presented in **Table 23**. The established NOEC values in these tests are nearly identical to the EC10 values for the algal test as shown in **Table 19**. As high densities of algal cells are introduced to the rotifers as food one may wonder whether this would mask the toxic effect of TRO. Therefore rotifers were also tested without addition of algae with water from test cycle 5. These individuals were inspected after 2 hours. There was no observed mortality even at 100 % treated ballast water.

Table 23. Test results of toxicity testing of ballast water treated with the CleanBallast treatment system on day 5 after deballasting using the oyster embryo test.

Chronic rotifer tests					
Test Cycle	**Test id**	**Test sample**	**Result EC_{50} (%)**	**Result NOEC (%)**	**Comment**
5	Chronic *B. plicatilis*	02.10.08	> 100	56	
9	Chronic *B. plicatilts*	22.10.08	> 100	>100	

Concluding remarks with respect to toxicity

Ballast water treated with the RWO CleanBallast treatment system was tested with 6 different marine species covering 3 trophic levels and 5 phyla. A total of 40 toxicity tests have been performed. The results from the tests showed a remarkable uniformity with respect to degree of toxicity across species and phyla. The algal tests gave the best picture of the variability in toxicity. There is a fairly good linear relationship with measured TRO in treated ballast water and observed toxicity. The algal tests showed that algae are equally or more sensitive to treated ballast water than other test species.

The measured effect concentrations in the algal tests showed generally higher toxicity in test cycles with seawater than in test cycles with brackish water. In the seawater tests, the EC_{10} varied from 14 to 56 % (average 33 %) and EC_{50} from 19 to 78 % (average 44 %) when excluding test 11, day 5. In test cycle 11, day 5, the TRO was neutralized by addition of sodium thiosulphate and EC_{10} and EC_{50} were both >100%, showing that the neutralization step efficiently removed the toxicity. In all test cycles with full seawater, the algae growth inhibition tests showed higher toxicity on day 5 than on day 0. The average EC_{50} values were 57 % on day 0 and 30 % on day 5. For brackish water

there was not a similar consistency of higher toxicity on day 5 compared with day 0. For brackish water the EC_{50} was always >100 % and EC_{10} was only occasionally less than 100 % on both day 0 and day 5.

The algal test proved to be more or equally sensitive as other test perfomed, it is therefore relevant to use the EC10 values of the algal tests for derivation of an aquatic PNEC in an environmental risk assessment.

4. References

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5. Appendix 1 - Disinfection by-product analyses

Disinfection by-products	Unit	Detection limit	Influent (WST)	Treated		Control	
Time (day)			0	0	5 (after deballasting)	0	5 (after deballasting)
Parameter							
Test Cycle 1 (Salinity >32 PSU)							
Trichloromethane Cl3CH	µg/l	0.5	<0.5	<0.5	< 0.5	<0.5	<0.50
Dichlorobromomethane CHBrCl2			<0.5	<0.5	< 0.5	<0.5	<0.50
Dibromochloromethane CHClBr2			<0.5	**4.3**	**8.2**	<0.5	<0.50
Tribromomethane Br3CH			<0.5	**61.0**	**150.0**	<0.5	**3.5**
Monochloroacetic acid (MCAA)*	µg/l	0.1-0.5	<0.5	<0.5	< 0.5	<0.5	<0.50
Dichloroacetic acid (DCAA)*			<0.3	<0.3	< 0.3	<0.3	**0.49**
Trichloroacetic acid (TCAA)*			<0.20	**0.28**	< 0.2	<0.20	<0.20
Monobromoacetic acid (MBAA)*			<0.20	<0.20	< 0.2	<0.20	<0.20
Dibromoacetic acid (DBAA)*			<0.10	<0.10	**8.3**	<0.10	**0.1**
Bromochloroacetic acid (BCAA)*			<0.10	<0.10	**0.32**	<0.10	<0.10
Dichlorobromoacteic acid (DCBAA)*			<0.10	<0.10	< 0.10	<0.10	<0.10
Dibromochloroacetic acid (DBCAA)*			<0.10	<0.10	< 0.10	<0.10	<0.10
Tribromoacteic acid (TBAA)*			<0.10	<0.10	< 0.10	<0.10	<0.10
2,4-dibromophenol*	µg/l	0.1	<0.10	<0.10	< 0.10	<0.10	<0.10
2,6-dibromophenol*			<0.10	<0.10	< 0.10	<0.10	<0.10
2,4,6-tribromophenol*			<0.10	<0.10	< 0.10	<0.10	<0.10
1,2-dibromoethane	µg/l	0.5	<0.5	<0.5	< 0.50	<0.5	<0.50
1.2.3-trichloropropane		1.0	<1.0	**1.0**	< 1.0	<1.0	<1.0
2-chlorotoluene	µg/l	0.5	<0.5	<0.5	< 0.50	<0.5	<0.50
4-chlorotoluene			<0.5	<0.5	< 0.50	<0.5	<0.50
1,2-dibromo-3-chloropropane	µg/l	0.5-1.0	<0.5	<1.0	< 1.0	<0.5	< 1.0
Tribromobenzene	only qualitativ analysis		Not detected	Not detected	Not detected	Not detected	Not detected
1,3,5-Tribrombensen	µg/l	0.1-0.5	<1.0	<1.0	<1.0	<1.0	<1.0
AOX (Adsorbable organically bound halogens)*	mg/l	0.01	<0.01	**0.029**	**0.05**	<0.01	<0.010
EOX (Extractable organohalogen compounds)			<0.01	<0.01	**0.026**	<0.01	<0.010
Bromate (BrO3)	µg/l	1.0	<1.0	**2.7**	**16**	<1.0	<1.0

Test Cycle 2 (Salinity >32 PSU)							
Trichloromethane Cl3CH	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichlorobromomethane CHBrCl2			<0.50	<0.50	<0.50	<0.50	<0.50
Dibromochloromethane CHClBr2			<0.50	**1.1**	**6.5**	<0.50	<0.50
Tribromomethane Br3CH			**0.88**	**42**	**190**	<0.50	**4**
Monochloroacetic acid (MCAA)*	µg/l	0.1-0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichloroacetic acid (DCAA)*			**0.39**	**0.97**	<0.30	**0.43**	<0.30
Trichloroacetic acid (TCAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Monobromoacetic acid (MBAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Dibromoacetic acid (DBAA)*			<0.10	**0.71**	**9.3**	<0.10	**0.43**
Bromochloroacetic acid (BCAA)*			<0.10	<0.10	**0.43**	<0.10	<0.10
Dichlorobromoacteic acid (DCBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Dibromochloroacetic acid (DBCAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Tribromoacteic acid (TBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4-dibromophenol*	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,6-dibromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4,6-tribromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10
1,2-dibromoethane	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
1,2,3-trichloropropane		1.0	<1.0	<1.0	<1.0	<1.0	<1.0
2-chlorotoluene	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
4-chlorotoluene			<0.50	<0.50	<0.50	<0.50	<0.50
1,2-dibromo-3-chloropropane	µg/l	1	<1.0	< 1.0	<1.0	< 1.0	<1.0
Tribrombenzene	only qualitativ analysis		Not detected	Not detected	Not detected	Not detected	Not detected
1,3,5-Tribrombensen	µg/l	0.1-0.5	<1.0	<1.0	<1.0	<1.0	<1.0
1-bromodimethylbenzene	only qualitativ analysis		-	**Detected**	-	-	-
AOX (Adsorbable organically bound halogens)*	mg/l	0.01	<0.010	**0.03**	**0.07**	<0.010	<0.010
EOX (Extractable organohalogen compounds)			<0.010	<0.010	**0.061**	<0.010	<0.010
Bromate (BrO3)	µg/l	1.0	<1.0	**9.70**	**19**	<1.0	<1.0

Test Cycle 3 (Salinity >32 PSU)							
Trichloromethane Cl3CH	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichlorobromomethane CHBrCl2			<0.50	<0.50	<0.50	<0.50	<0.50
Dibromochloromethane CHClBr2			<0.50	**0.98**	**6.3**	<0.50	<0.50
Tribromomethane Br3CH			<0.50	**27**	**130**	<0.50	**0.52**
Monochloroacetic acid (MCAA)*	µg/l	0.1-0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichloroacetic acid (DCAA)*			<0.30	<0.30	<0.30	<0.30	<0.30
Trichloroacetic acid (TCAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Monobromoacetic acid (MBAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Dibromoacetic acid (DBAA)*			<0.10	**0.54**	**2.7**	**0.22**	**0.12**
Bromochloroacetic acid (BCAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Dichlorobromoacteic acid (DCBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Dibromochloroacetic acid (DBCAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Tribromoacteic acid (TBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4-dibromophenol*	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,6-dibromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4,6-tribromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10
1,2-dibromoethane	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
1,2,3-trichloropropane		1.0	<1.0	<1.0	<1.0	<1.0	<1.0
2-chlorotoluene	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
4-chlorotoluene			<0.50	<0.50	<0.50	<0.50	<0.50
1,2-dibromo-3-chloropropane	µg/l	1	<1.0	<1.0	<1.0	<1.0	<1.0
Tribrombenzene	only qualitativ analysis		Not detected	Not detected		Not detected	
1,3,5-Tribrombensen	µg/l	0.1-0.5	<1.0	<1.0	<1.0	<1.0	<1.0
1-bromodimethylbenzene	only qualitativ analysis		Not detected	Not detected		Not detected	
AOX (Adsorbable organically bound halogens)*	mg/l	0.01	<0.010	**0.03**	**0.07**	**0.01**	**0.02**
EOX (Extractable organohalogen compounds)			<0.010	<0.010	**0.029**	<0.010	<0.010
Bromate (BrO3)	µg/l	1.0	<1.0	**5.7**	**8.5**	<1.0	<1.0

Test Cycle 4 (Salinity >32 PSU)							
Trichloromethane Cl3CH	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichlorobromomethane CHBrCl2			<0.50	<0.50	<0.50	<0.50	<0.50
Dibromochloromethane CHClBr2			<0.50	<0.50	**4.3**	<0.50	<0.50
Tribromomethane Br3CH			<0.50	**34**	**86**	<0.50	<0.50
Monochloroacetic acid (MCAA)*	µg/l	0.1-0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichloroacetic acid (DCAA)*			<0.30	<0.30	<0.30	<0.30	<0.30
Trichloroacetic acid (TCAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Monobromoacetic acid (MBAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Dibromoacetic acid (DBAA)*			<0.10	**1.1**	**3.4**	<0.10	**0.32**
Bromochloroacetic acid (BCAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Dichlorobromoacteic acid (DCBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Dibromochloroacetic acid (DBCAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Tribromoacteic acid (TBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4-dibromophenol*	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,6-dibromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4,6-tribromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10
1,2-dibromoethane	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
1,2,3-trichloropropane		1.0	<1.0	<1.0	<1.0	<1.0	<1.0
2-chlorotoluene	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
4-chlorotoluene			<0.50	<0.50	<0.50	<0.50	<0.50
1,2-dibromo-3-chloropropane	µg/l	1	<1.0	<1.0	<1.0	<1.0	<1.0
Dimethylbromobensen	only qualitativ analysis		-	-	**Detected**	-	-
Tribromobensen	only qualitativ analysis		Not detected	Not detected	Not detected	Not detected	Not detected
1,3,5-Tribrombensen	µg/l	0.1-0.5	<1.0	<1.0	<1.0	<1.0	<1.0
*AOX (Adsorbable organically bound halogens)**	mg/l	0.01	0.01	**0.01**	<0.010	<0.010	<0.010
EOX (Extractable organohalogen compounds)			<0.010	<0.010	<0.010	<0.010	<0.010
Bromate (BrO3)	µg/l	1.0	<1.0	**4.0**	**6.0**	<1.0	<1.0

Test Cycle 5 (Salinity >32 PSU)							
Trichloromethane Cl3CH	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichlorobromomethane CHBrCl2			<0.50	<0.50	<0.50	<0.50	<0.50
Dibromochloromethane CHClBr2			<0.50	**0.74**	**4.8**	<0.50	<0.50
Tribromomethane Br3CH			<0.50	**25**	**98**	<0.50	**1.3**
Monochloroacetic acid (MCAA)*	µg/l	0.1-0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichloroacetic acid (DCAA)*			<0.30	<0.30	<0.30	<0.30	<0.30
Trichloroacetic acid (TCAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Monobromoacetic acid (MBAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Dibromoacetic acid (DBAA)*			<0.10	**1.7**	**5.6**	<0.10	<0.10
Bromochloroacetic acid (BCAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Dichlorobromoacteic acid (DCBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Dibromochloroacetic acid (DBCAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Tribromoacteic acid (TBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4-dibromophenol*	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,6-dibromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4,6-tribromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10
1,2-dibromoethane	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
1,2,3-trichloropropane		1.0	<1.0	<1.0	<1.0	<1.0	<1.0
2-chlorotoluene	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
4-chlorotoluene			<0.50	<0.50	<0.50	<0.50	<0.50
1,2-dibromo-3-chloropropane	µg/l	1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Dimethylbromobensen	only qualitativ analysis		-	-	**Detected**	**Detected**	-
Tribromobensen	only qualitativ analysis		Not detected	Not detected	Not detected	Not detected	Not detected
1,3,5-Tribrombensen	µg/l	0.1-0.5	<1.0	<1.0	<1.0	<1.0	<1.0
AOX (Adsorbable organically bound halogens)*	mg/l	0.01	<0.010	**0.01**	**0.05**	<0.010	<0.010
EOX (Extractable organohalogen compounds)			<0.010	<0.010	**0.018**	<0.010	<0.010
Bromate (BrO3)	µg/l	1.0	<1.0	**3.8**	**6.0**	<1.0	<1.0

Test Cycle 6 (Salinity <22 PSU)							
Trichloromethane Cl3CH	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichlorobromomethane CHBrCl2			<0.50	<0.50	<0.50	<0.50	<0.50
Dibromochloromethane CHClBr2			<0.50	**3.8**	**6**	<0.50	<0.50
Tribromomethane Br3CH			<0.50	**89**	**110**	<0.50	<1.1
Monochloroacetic acid (MCAA)*	µg/l	0.1-0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichloroacetic acid (DCAA)*			<0.30	<0.30	<0.30	<0.30	<0.30
Trichloroacetic acid (TCAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Monobromoacetic acid (MBAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Dibromoacetic acid (DBAA)*			<0.10	**7.8**	**4.9**	<0.10	<0.10
Bromochloroacetic acid (BCAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Dichlorobromoacteic acid (DCBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Dibromochloroacetic acid (DBCAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Tribromoacteic acid (TBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4-dibromophenol*	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,6-dibromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4,6-tribromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10
1,2-dibromoethane	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
1,2,3-trichloropropane		1.0	<1.0	<1.0	<1.0	<1.0	<1.0
2-chlorotoluene	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
4-chlorotoluene			<0.50	<0.50	<0.50	<0.50	<0.50
1,2-dibromo-3-chloropropane	µg/l	1.0	<1.0	<1.0	<1.0	<1.0	<1.0
1,2,3-Tribrombensen	only qualitativ analysis		Not detected	Not detected	Not detected	Not detected	Not detected
1,3,5-Tribrombensen	µg/l	0.1-0.5	<1.0	<1.0	<1.0	<1.0	<1.0
AOX (Adsorbable organically bound halogens)*	mg/l	0.01	<0.010	**0.04**	**0.06**	<0.010	<0.010
EOX (Extractable organohalogen compounds)			<0.010	<0.010	0.011	<0.010	<0.010
Bromate (BrO3)	µg/l	1.0	<1.0	**15.0**	**27.0**	<1.0	<1.0

Test Cycle 7 (Salinity <22 PSU)							
Trichloromethane Cl3CH	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichlorobromomethane CHBrCl2			<0.50	<0.50	<0.50	<0.50	<0.50
Dibromochloromethane CHClBr2			<0.50	**3.4**	**7.7**	<0.50	<0.50
Tribromomethane Br3CH			<0.50	**80**	**240**	<0.50	**1.3**
Monochloroacetic acid (MCAA)*	µg/l	0.1-0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichloroacetic acid (DCAA)*			<0.30	<0.30	<0.30	<0.30	<0.30
Trichloroacetic acid (TCAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Monobromoacetic acid (MBAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Dibromoacetic acid (DBAA)*			<0.10	**0.9**	**17**	<0.10	**0.13**
Bromochloroacetic acid (BCAA)*			<0.10	<0.10	**1.1**	<0.10	<0.10
Dichlorobromoacteic acid (DCBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Dibromochloroacetic acid (DBCAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Tribromoacteic acid (TBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4-dibromophenol*	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,6-dibromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4,6-tribromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10
1,2-dibromoethane	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
1,2,3-trichloropropane		1.0	<1.0	<1.0	<1.0	<1.0	<1.0
2-chlorotoluene	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
4-chlorotoluene			<0.50	<0.50	<0.50	<0.50	<0.50
1,2-dibromo-3-chloropropane	µg/l	1.0	<1.0	<1.0	<1.0	<1.0	<1.0
1,2,3-Tribrombensen	only qualitativ analysis		Not detected	Not detected	Not detected	Not detected	Not detected
1,3,5-Tribrombensen	µg/l	0.1-0.5	<1.0	<1.0	-	<1.0	-
AOX (Adsorbable organically bound halogens)*	mg/l	0.01	<0.010	**0.05**	**0.07**	<0.010	<0.010
EOX (Extractable organohalogen compounds)			<0.010	<0.010	0.015	<0.010	<0.010
Bromate (BrO3)	µg/l	1.0	<1.0	**14**	**24**	<1.0	<1.0

Test Cycle 8 (Salinity <22 PSU)							
Trichloromethane Cl3CH	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichlorobromomethane CHBrCl2			<0.50	<0.50	<0.50	<0.50	<0.50
Dibromochloromethane CHClBr2			<0.50	**3.9**	**6.9**	<0.50	<0.50
Tribromomethane Br3CH			<0.50	**120**	**150**	<0.50	**0.96**
Monochloroacetic acid (MCAA)*	µg/l	0.1-0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichloroacetic acid (DCAA)*			<0.30	<0.30	<0.30	<0.30	<0.30
Trichloroacetic acid (TCAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Monobromoacetic acid (MBAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Dibromoacetic acid (DBAA)*			<0.10	**9.1**	**3.1**	<0.10	<0.10
Bromochloroacetic acid (BCAA)*			<0.10	**0.82**	**0.18**	<0.10	<0.10
Dichlorobromoacteic acid (DCBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Dibromochloroacetic acid (DBCAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Tribromoacteic acid (TBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4-dibromophenol*	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,6-dibromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4,6-tribromophenol*			<0.10	<0.10	**0.39**	<0.10	<0.10
1,2-dibromoethane	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
1,2,3-trichloropropane		1.0	<1.0	<1.0	<1.0	<1.0	<1.0
2-chlorotoluene	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
4-chlorotoluene			<0.50	<0.50	<0.50	<0.50	<0.50
1,2-dibromo-3-chloropropane	µg/l	1.0	<1.0	<1.0	<1.0	<1.0	<1.0
1,2,3-Tribrombensen	only qualitativ analysis		Not detected	Not detected	Not detected	Not detected	Not detected
AOX (Adsorbable organically bound halogens)*	mg/l	0.01	<0.010	**0.06**	**0.07**	<0.010	<0.010
EOX (Extractable organohalogen compounds)			<0.010	<0.010	**0.015**	<0.010	<0.010
Bromate (BrO3)	µg/l	1.0	<1.0	**13**	**27**	<1.0	<1.0

Test Cycle 9 (Salinity <22 PSU)							
Trichloromethane Cl3CH	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichlorobromomethane CHBrCl2			<0.50	<0.50	<0.50	<0.50	<0.50
Dibromochloromethane CHClBr2			<0.50	**3.7**	**3.5**	<0.50	<0.50
Tribromomethane Br3CH			<0.50	**96**	**96**	<0.50	<0.50
Monochloroacetic acid (MCAA)*	µg/l	0.1-0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichloroacetic acid (DCAA)*			<0.30	<0.30	<0.30	<0.30	<0.30
Trichloroacetic acid (TCAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Monobromoacetic acid (MBAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Dibromoacetic acid (DBAA)*			<0.10	**1.4**	**2.2**	<0.10	0,31
Bromochloroacetic acid (BCAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Dichlorobromoacteic acid (DCBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Dibromochloroacetic acid (DBCAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Tribromoacteic acid (TBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4-dibromophenol*	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,6-dibromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4,6-tribromophenol*			<0.10	**1.9**	**0.52**	<0.10	<0.10
1,2-dibromoethane	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
1,2,3-trichloropropane		1.0	<1.0	<1.0	<1.0	<1.0	<1.0
2-chlorotoluene	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
4-chlorotoluene			<0.50	<0.50	<0.50	<0.50	<0.50
1,2-dibromo-3-chloropropane	µg/l	1.0	<1.0	<1.0	<1.0	<1.0	<1.0
1,2,3-Tribrombensen	only qualitativ analysis		Not detected	Not detected	Not detected	Not detected	Not detected
AOX (Adsorbable organically bound halogens)*	mg/l	0.01	<0.010	**0.04**	**0.03**	<0.010	<0.010
EOX (Extractable organohalogen compounds)			<0.010	<0.010	<0.010	<0.010	<0.010
Bromate (BrO3)	µg/l	1.0	<1.0	**16**	**29**	<1.0	< 1.0

Test Cycle 10 (Salinity <22 PSU)							
Trichloromethane Cl3CH	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichlorobromomethane CHBrCl2			<0.50	<0.50	<0.50	<0.50	<0.50
Dibromochloromethane CHClBr2			<0.50	**5.1**	**7.6**	<0.50	<0.50
Tribromomethane Br3CH			<0.50	**86**	**140**	**0.5**	**1.4**
Monochloroacetic acid (MCAA)*	µg/l	0.1-0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichloroacetic acid (DCAA)*			<0.30	<0.30	<0.30	<0.30	<0.30
Trichloroacetic acid (TCAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Monobromoacetic acid (MBAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Dibromoacetic acid (DBAA)*			**0.28**	**49**	**120**	**0.48**	**1.7**
Bromochloroacetic acid (BCAA)*			<0.10	**0.74**	**1.5**	<0.10	<0.10
Dichlorobromoacteic acid (DCBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Dibromochloroacetic acid (DBCAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Tribromoacteic acid (TBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4-dibromophenol*	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,6-dibromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4,6-tribromophenol*			**0.52**	<0.10	<0.10	<0.10	<0.10
1,2-dibromoethane	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
1,2,3-trichloropropane		1.0	<1.0	<1.0	<1.0	<1.0	<1.0
2-chlorotoluene	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
4-chlorotoluene			<0.50	<0.50	<0.50	<0.50	<0.50
1,2-dibromo-3-chloropropane	µg/l	1.0	<1.0	<1.0	<1.0	<1.0	<1.0
1,2,3,-Tribromobensen	only qualitativ analysis		not detected	not detected	not detected	not detected	not detected
1,3,5 - Tribromobensen	µg/l	1	<1.0	<1.0	<1.0	<1.0	<1.0
AOX (Adsorbable organically bound halogens)*	mg/l	0.01	<0.010	**0.04**	**0.06**	<0.010	<0.010
EOX (Extractable organohalogen compounds)			<0.010	<0.010	0.01	<0.010	<0.010
Bromate (BrO3)	µg/l	1.0	<1.0	**18**	**25**	<1.0	< 1.0

Test Cycle 11 (Salinity >32 PSU)							
Trichloromethane Cl3CH	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichlorobromomethane CHBrCl2			<0.50	<0.50	<0.50	<0.50	<0.50
Dibromochloromethane CHClBr2			<0.50	**1.8**	**4**	<0.50	<0.50
Tribromomethane Br3CH			<0.50	**37**	**130**	<0.50	<0.50
Monochloroacetic acid (MCAA)*	µg/l	0.1-0.5	<0.50	<0.50	*******	<0.50	*******
Dichloroacetic acid (DCAA)*			<0.30	<0.30	*******	<0.30	*******
Trichloroacetic acid (TCAA)*			<0.20	<0.20	*******	<0.20	*******
Monobromoacetic acid (MBAA)*			<0.20	<0.20	*******	<0.20	*******
Dibromoacetic acid (DBAA)*			**0.31**	**33**	*******	1	*******
Bromochloroacetic acid (BCAA)*			<0.10	**0.43**	*******	0.1	*******
Dichlorobromoacteic acid (DCBAA)*			<0.10	<0.10	*******	<0.10	*******
Dibromochloroacetic acid (DBCAA)*			<0.10	<0.10	*******	<0.10	*******
Tribromoacteic acid (TBAA)*			<0.10	<0.10	*******	<0.10	*******
2,4-dibromophenol*	µg/l	0.1	<0.10	<0.10	*******	<0.10	*******
2,6-dibromophenol*			<0.10	<0.10	*******	<0.10	*******
2,4,6-tribromophenol*			<0.10	<0.10	*******	<0.10	*******
1,2-dibromoethane	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
1,2,3-trichloropropane		1.0	<1.0	<1.0	<1.0	<1.0	<1.0
2-chlorotoluene	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
4-chlorotoluene			<0.50	<0.50	<0.50	<0.50	<0.50
1,2-dibromo-3-chloropropane	µg/l	1.0	<1.0	<1.0	<1.0	<1.0	<1.0
1,2,3-Tribromobensen	only qualitativ analysis		not detected	not detected	note	not detected	note
1,3,5-Tribromobensen	µg/l	1	<1.0	<1.0	<1.0	<1.0	<1.0
*AOX (Adsorbable organically bound halogens)**	mg/l	0.01	<0.010	**0.02**	**0.06**	<0.010	<0.010
EOX (Extractable organohalogen compounds)			<0.010	<0.010	<0.010	<0.010	<0.010
Bromate (BrO3)	µg/l	1.0	< 1.0	**4.5**	**6.9**	< 1.0	< 1.0

Test Cycle 12 (Salinity >32 PSU)							
Trichloromethane Cl3CH	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichlorobromomethane CHBrCl2			<0.50	<0.50	<0.50	<0.50	<0.50
Dibromochloromethane CHClBr2			<0.50	**1.2**	**4.8**	<0.50	<0.80
Tribromomethane Br3CH			<0.50	**53**	**170**	<0.50	**0.82**
Monochloroacetic acid (MCAA)*	µg/l	0.1-0.5	*******	*******	<0.50	*******	<0.50
Dichloroacetic acid (DCAA)*			*******	*******	<0.30	*******	<0.30
Trichloroacetic acid (TCAA)*			*******	*******	<0.20	*******	<0.20
Monobromoacetic acid (MBAA)*			*******	*******	<0.20	*******	<0.20
Dibromoacetic acid (DBAA)*			*******	*******	**4.2**	*******	**0.13**
Bromochloroacetic acid (BCAA)*			*******	*******	<0.10	*******	<0.10
Dichlorobromoacteic acid (DCBAA)*			*******	*******	<0.10	*******	<0.10
Dibromochloroacetic acid (DBCAA)*			*******	*******	<0.10	*******	<0.10
Tribromoacteic acid (TBAA)*			*******	*******	<0.10	*******	<0.10
2,4-dibromophenol*	µg/l	0.1	*******	*******	<0.10	*******	<0.10
2,6-dibromophenol*			*******	*******	<0.10	*******	<0.10
2,4,6-tribromophenol*			*******	*******	<0.10	*******	<0.10
1,2-dibromoethane	µg/l	0.5	< 0.50	<0.50	<0.50	<0.50	<0.50
1,2,3-trichloropropane		1.0	<1.0	<1.0	<1.0	<1.0	<1.0
2-chlorotoluene	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
4-chlorotoluene			<0.50	<0.50	<0.50	<0.50	<0.50
1,2-dibromo-3-chloropropane	µg/l	1.0	<1.0	<1.0	<1.0	<1.0	<1.0
1,2,3-Tribromobensen	only qualitativ analysis		note	note	note	note	note
1,3,5-Tribromobensen	µg/l	1	<1.0	<1.0	<1.0	<1.0	<1.0
AOX (Adsorbable organically bound halogens)*	mg/l	0.01	<0.010	**0.02**	**0.05**	<0.010	*******
EOX (Extractable organohalogen compounds)			<0.010	<0.010	0.014	<0.010	<0.010
Bromate (BrO3)	µg/l	1.0	< 1.0	**4.9**	**7.7**	< 1.0	< 1.0

Test Cycle 13 (Salinity >32 PSU)							
Trichloromethane Cl3CH	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichlorobromomethane CHBrCl2			<0.50	<0.50	<0.50	<0.50	<0.50
Dibromochloromethane CHClBr2			<0.50	**1.3**	**5.6**	<0.50	<0.50
Tribromomethane Br3CH			<0.50	**47**	**200**	<0.50	**1.5**
Monochloroacetic acid (MCAA)*	µg/l	0.1-0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichloroacetic acid (DCAA)*			<0.30	<0.30	<0.30	<0.30	<0.30
Trichloroacetic acid (TCAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Monobromoacetic acid (MBAA)*			<0.20	<0.20	<0.20	<0.20	<0.20
Dibromoacetic acid (DBAA)*			<0.10	**0.37**	**1.3**	<0.10	<0.10
Bromochloroacetic acid (BCAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Dichlorobromoacteic acid (DCBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Dibromochloroacetic acid (DBCAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
Tribromoacteic acid (TBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4-dibromophenol*	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,6-dibromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10
2,4,6-tribromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10
1,2-dibromoethane	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
1,2,3-trichloropropane		1.0	<1.0	<1.0	<1.0	<1.0	<1.0
2-chlorotoluene	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
4-chlorotoluene			<0.50	<0.50	<0.50	<0.50	<0.50
1,2-dibromo-3-chloropropane	µg/l	1.0	<1.0	<1.0	<1.0	<1.0	<1.0
1,2,3-Tribromobensen	only qualitativ analysis		note	note	note	note	note
1,3,5-Tribromobensen	µg/l	1	<1.0	<1.0	<1.0	<1.0	<1.0
AOX (Adsorbable organically bound halogens)*	mg/l	0.01	<0.010	*******	**0.06**	<0.010	**0.02**
EOX (Extractable organohalogen compounds)			<0.010	<0.010	**0.014**	<0.010	<0.010
Bromate (BrO3)	µg/l	1.0	< 1.0	**5.3**	**7.0**	< 1.0	< 1.0

* not accredited analysis

6. Appendix 2 – Chemical fate study of disinfection by-products.

Table 1. Chemical fate study of disinfection by-products in treated seawater

Disinfection by-products by ALS Scandinavia	**Unit**	**Detection limit**	**Influent**	**Treated water**									
Test Cycle 2 (Salinity >32 PSU) decay test				**After first treatment**			**After second treatment**						
Time (day, hours)			**0**	**0**	**2**	**5**	**0**	**0,5h**	**1h**	**2h**	**4h**	**24h**	**48h**
Parameter													
Trichloromethane Cl3CH	µg/l	0,5	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
Dichlorobromomethane CHBrCl2			<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
Dibromochloromethane CHClBr2			<0.50	**1,1**	**5,6**	**7,9**	**6,5**	**6,6**	**6,2**	**6,7**	**5,2**	**6,2**	**6,9**
Tribromomethane Br3CH			**0,88**	**42**	**170**	**190**	**190**	**180**	**200**	**180**	**120**	**150**	**170**
Monochloroacetic acid (MCAA)*	µg/l	0,1-0,5	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
Dichloroacetic acid (DCAA)*			**0,39**	**0,97**	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30
Trichloroacetic acid (TCAA)*			<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
Monobromoacetic acid (MBAA)*			<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
Dibromoacetic acid (DBAA)*			<0.10	**0,71**	**9,7**	**8,4**	**9,3**	**7,7**	**10**	**6,7**	**22**	**20**	**29**
Bromochloroacetic acid (BCAA)*			<0.10	<0.10	**0,29**	**0,28**	**0,43**	**0,29**	**0,39**	**0,29**	**0,49**	**0,41**	**0,88**
Dichlorobromoacteic acid (DCBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Dibromochloroacetic acid (DBCAA)*			<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Tribromoacteic acid (TBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
2,4-dibromophenol*	µg/l	0,1	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
2,6-dibromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
2,4,6-tribromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
1,2-dibromoethane	µg/l	0,5	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
1,2,3-trichloropropane		1,0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
2-chlorotoluene	µg/l	0,5	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
4-chlorotoluene			<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
1,2-dibromo-3-chloropropane	µg/l	1,0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
1,3,5-Tribrombensen	µg/l	0,1-0,5	<1,0	<1,0	<1,0	<1,0	<1,0	<1,0	<1,0	<1,0	<1,0	<1,0	<1,0
Tribromobenzene	only qualitativ analysis		none	none	none	none	none	none	none	none	none	none	none
1-bromodimethylbenzene			none	**detected**	none	none	none	none	none	none	**detected**	**detected**	**detected**
Bromomethylbenze			none	**none**	none	none	none	none	none	none	**traces**	**traces**	**traces**
AOX (Adsorbable organically bound halogens)*	mg/l	0,01	<0.010	**0,03**	**0,05**	**0,11**	**0,07**	**0,07**	**0,05**	**0,07**	**0,09**	**0,08**	**0,1**
EOX (Extractable organohalogen compounds)			<0.010	<0.010	**0,046**	**0,051**	**0,061**	**0,061**	**0,014**	**0,045**	**0,082**	**0,062**	**0,029**
Bromate (BrO3)	µg/l	1,0	<1,0	**9,7**	**11**	**11**	**19**	**20**	**20**	**20**	**22**		**23**

*not accreditated analysis

Table 2. Chemical fate study of disinfection by-products in control seawater

Disinfection by-products by ALS Scandinavia	**Unit**	**Detection limit**	**Influent**	**Control water**								
Test Cycle 2 (Salinity >32 PSU) decay test				**After first by-pass**			**After second by-pass**					
Time (day, hours)			**0**	**0**	**2**	**5**	**0**	**0,5h**	**2h**	**4h**	**24h**	**48h**
Parameter												
Trichloromethane Cl3CH	µg/l	0,5	<0.50	<0.50	<0.50	<0.50	<0.50	-	-	-	-	<0.50
Dichlorobromomethane CHBrCl2			<0.50	<0.50	<0.50	<0.50	<0.50	-	-	-	-	<0.50
Dibromochloromethane CHClBr2			<0.50	<0.50	<0.50	<0.50	<0.50	-	-	-	-	<0.50
Tribromomethane Br3CH			**0,88**	<0.50	**1,5**	<0.50	**4**	-	-	-	-	**3,7**
Monochloroacetic acid (MCAA)*	µg/l	0,1-0,5	<0.50	<0.50	<0.50	<0.50	<0.50	-	-	-	-	<0.50
Dichloroacetic acid (DCAA)*			**0,39**	**0,43**	<0.30	<0.30	<0.30	-	-	-	-	<0.30
Trichloroacetic acid (TCAA)*			<0.20	<0.20	<0.20	<0.20	<0.20	-	-	-	-	<0.20
Monobromoacetic acid (MBAA)*			<0.20	<0.20	<0.20	<0.20	<0.20	-	-	-	-	<0.20
Dibromoacetic acid (DBAA)*			<0.10	<0.10	**1,7**	**11**	**0,43**	-	-	-	-	**0,22**
Bromochloroacetic acid (BCAA)*			<0.10	<0.10	<0.10	**0,42**	<0.10	-	-	-	-	<0.10
Dichlorobromoacteic acid (DCBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10	-	-	-	-	<0.10
Dibromochloroacetic acid (DBCAA)*			<0.10	<0.10	<0.10	<0.10	<0.10	-	-	-	-	<0.10
Tribromoacteic acid (TBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10	-	-	-	-	<0.10
2,4-dibromophenol*	µg/l	0,1	<0.10	<0.10	<0.10	<0.10	<0.10	-	-	-	-	<0.10
2,6-dibromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10	-	-	-	-	<0.10
2,4,6-tribromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10	-	-	-	-	<0.10
1,2-dibromoethane	µg/l	0,5	<0.50	<0.50	<0.50	<0.50	<0.50	-	-	-	-	<0.50
1,2,3-trichloropropane		1,0	<1.0	<1.0	<1.0	<1.0	<1.0	-	-	-	-	<1.0
2-chlorotoluene	µg/l	0,5	<0.50	<0.50	<0.50	<0.50	<0.50	-	-	-	-	<0.50
4-chlorotoluene			<0.50	<0.50	<0.50	<0.50	<0.50	-	-	-	-	<0.50
1,2-dibromo-3-chloropropane	µg/l	1,0	<1.0	<1.0	<1.0	<1.0	<1.0	-	-	-	-	<1.0
1,3,5-Tribrombensen	µg/l	0,1-0,5	<1,0	<1,0	<1,0	<1,0	<1,0	-	-	-	-	<1,0
Tribromobenzene	only qualitativ analysis		none	none	none	none	none	-	-	-	-	none
1-bromodimethylbenzene			none	none	none	none	none	-	-	-	-	none
Bromomethylbenze			none	none	none	none	none					none
AOX (Adsorbable organically bound halogens)*	mg/l	0,01	<0.010	<0.010	<0.010	<0.010	<0.010	-	-	-	-	<0.010
EOX (Extractable organohalogen compounds)			<0.010	<0.010	<0.010	<0.010	<0.010	-	-	-	-	**0,032**
Bromate (BrO3)	µg/l	1,0	<1,0	<1,0	<1,0	<1,0	<1,0	-	-	-	-	<1,0

*not accreditated analysis

Table 3. Chemical fate study of disinfection by-products in treated brackish water

	Unit	Detection limit	Influent	Treated water									
Test Cycle 9 (Salinity <22 PSU) decay test				**After first treatment**			**After second treatment**						
Time (day, hours)			**0**	**0**	**2**	**5**	**0**	**0,5h**	**1h**	**2h**	**4h**	**24h**	**48h**
Parameter													
Trichloromethane Cl3CH	µg/l	0,5	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
Dichlorobromomethane CHBrCl2			<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	0	<0.50
Dibromochloromethane CHClBr2			<0.50	**3,7**	**3,5**	**3,5**	**6,1**	**6,6**	**7**	**7,3**	**7,7**	**9,5**	**8,9**
Tribromomethane Br3CH			<0.50	**96**	**100**	**96**	**160**	**220**	**210**	**220**	**220**	**180**	**170**
Monochloroacetic acid (MCAA)*	µg/l	0,1-0,5	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
Dichloroacetic acid (DCAA)*			<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30
Trichloroacetic acid (TCAA)*			<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
Monobromoacetic acid (MBAA)*			<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
Dibromoacetic acid (DBAA)*			<0.10	**1,4**	**3,9**	**2,2**	**7,1**	**7,2**	**11**	**8,5**	**11**	**61**	**8**
Bromochloroacetic acid (BCAA)*			<0.10	<0.10	**0,31**	<0.10	**0,42**	**0,4**	**0,92**	**0,71**	**1,2**	**5**	<0.10
Dichlorobromoacteic acid (DCBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Dibromochloroacetic acid (DBCAA)*			<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Tribromoacteic acid (TBAA)*			<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
2,4-dibromophenol*	µg/l	0,1	<0.10	<0.10	<0.10	<0.10	<0.10	**0,14**	<0.10	<0.10	**0,1**	<0.10	**2,7**
2,6-dibromophenol*			<0.10	<0.10	<0.10	<0.10	<0.10	**0,11**	<0.10	<0.10	<0.10	<0.10	**1,7**
2,4,6-tribromophenol*			<0.10	**1,9**	**0,92**	**0,52**	**0,13**	**0,25**	**0,15**	**0,21**	**0,32**	**<0.97**	**3**
1,2-dibromoethane	µg/l	0,5	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
1,2,3-trichloropropane		1,0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
2-chlorotoluene	µg/l	0,5	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
4-chlorotoluene			<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
1,2-dibromo-3-chloropropane	µg/l	1,0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
1,2,3-Tribrombensen	only qualitativ analysis		none	none	none	none	none	none	none	none	none	none	none
1,3,5 -Tribrombenzen					<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
AOX (Adsorbable organically bound halogens)*	mg/l	0,01	<0.010	**0,04**	**0,03**	**0,03**	**0,07**	**0,06**	**0,06**	**0,06**	**0,04**	**0,1**	**0,08**
EOX (Extractable organohalogen compounds)			<0.010	<0.010	<0.010	<0.010	**0,012**	**0,016**	**0,014**	**0,013**	**0,014**	**0,011**	<0.010
Bromate (BrO3)	µg/l	1,0	<1,0	**16**	**17**	**16**	**29**	**36**	**34**	**32**	**31**	**28**	**27**

*not accreditated analysis

Table 4. Chemical fate study of disinfection by-products in control brackish water

	Unit	Detection limit	Control water								
Test Cycle 9 (Salinity <22 PSU) decay test			**After first by-pass**			**After second by-pass**					
Time (day, hours)			**0**	**2**	**5**	**0**	**0,5h**	**2h**	**4h**	**24h**	**48h**
Parameter											
Trichloromethane Cl3CH	µg/l	0,5	<0.50	<0.50	<0.50	<0.50	-	-	-	-	<0.50
Dichlorobromomethane CHBrCl2			<0.50	<0.50	<0.50	<0.50	-	-	-	-	<0.50
Dibromochloromethane CHClBr2			<0.50	<0.50	<0.50	<0.50	-	-	-	-	<0.50
Tribromomethane Br3CH			<0.50	<0.50	<0.50	0,73	-	-	-	-	1,9
Monochloroacetic acid (MCAA)*	µg/l	0,1-0,5	<0.50	<0.50	<0.50	<0.50	-	-	-	-	<0.50
Dichloroacetic acid (DCAA)*			<0.30	<0.30	<0.30	<0.30	-	-	-	-	<0.30
Trichloroacetic acid (TCAA)*			<0.20	<0.20	<0.20	<0.20	-	-	-	-	<0.20
Monobromoacetic acid (MBAA)*			<0.20	<0.20	<0.20	<0.20	-	-	-	-	<0.20
Dibromoacetic acid (DBAA)*			<0.10	**0,22**	**0,31**	**0,22**	-	-	-	-	0,91
Bromochloroacetic acid (BCAA)*			<0.10	<0.10	<0.10	<0.10	-	-	-	-	<0.10
Dichlorobromoacteic acid (DCBAA)*			<0.10	<0.10	<0.10	<0.10	-	-	-	-	<0.10
Dibromochloroacetic acid (DBCAA)*			<0.10	<0.10	<0.10	<0.10	-	-	-	-	<0.10
Tribromoacteic acid (TBAA)*			<0.10	<0.10	<0.10	<0.10	-	-	-	-	<0.10
2,4-dibromophenol*	µg/l	0,1	<0.10	**0,13**	<0.10	<0.10	-	-	-	-	<0.10
2,6-dibromophenol*			<0.10	**0,1**	<0.10	<0.10	-	-	-	-	<0.10
2,4,6-tribromophenol*			<0.10	**0,1**	<0.10	<0.10	-	-	-	-	<0.10
1,2-dibromoethane	µg/l	0,5	<0.50	<0.50	<0.50	<0.50	-	-	-	-	<0.50
1,2,3-trichloropropane		1,0	<1.0	<1.0	<1.0	<1.0	-	-	-	-	<1.0
2-chlorotoluene	µg/l	0,5	<0.50	<0.50	<0.50	<0.50	-	-	-	-	<0.50
4-chlorotoluene			<0.50	<0.50	<0.50	<0.50	-	-	-	-	<0.50
1,2-dibromo-3-chloropropane	µg/l	1,0	<1.0	<1.0	<1.0	<1.0	-	-	-	-	<1.0
1,2,3-Tribrombensen	only qualitativ analysis		none	none	none	none					none
1,3,5 -Tribrombenzen				<1.0	<1.0	<1.0					<1.0
AOX (Adsorbable organically bound halogens)*	mg/l	0,01	<0.010	<0.010	<0.010	0,03	-	-	-	-	<0.010
EOX (Extractable organohalogen compounds)			<0.010	<0.010	<0.010	<0.010	-	-	-	-	<0.010
Bromate (BrO3)	µg/l	1,0	<1.0	<1.0	<1.0	<1.0	-	-	-	-	< 1,0

* not accreditated analysis

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